

Synthesis and characterization of photoswitchable building blocks based on spirobenzopyrans and new approaches for postsynthetic oligonucleotide labeling

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Dedicated to my family.

The most exciting phrase to hear in science,
the one that heralds new discoveries,
is not "Eureka!" ("I found it!")
but rather "hmm...that's funny..."

Isaac Asimov

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Chapter 1: Synthesis and optical properties of building blocks based on photochromic spirobenzopyrans and spiroxazines

1.1. Introduction

1.1.1. Definition of photochromism

Photochromism is a reversible transformation of a chemical species induced in one or both directions by absorption of light between two forms A and B, which have different absorption spectra. Literally, the term describes a photoinduced change in color (greek: *phos* = light, *chroma* = color). The difference of the two forms A and B in their optical properties is accompanied by different physical properties, such as refractive indices, dielectric constants, etc. At least one reversible process is induced by photoexcitation. The thermodynamically stable A is transformed into B by irradiation. The reverse reaction can occur photochemically (*P*-type photochromism) or thermally (*T*-type photochromism).^[1]

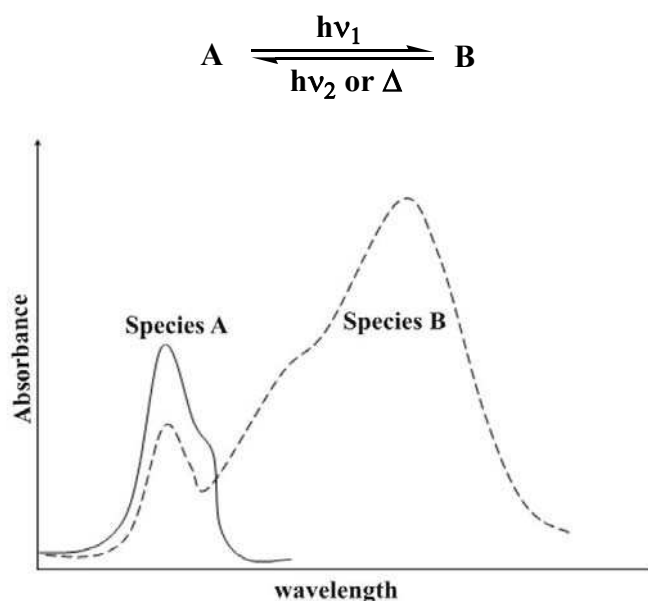


Figure 1.1. Absorption spectra for a typical AB photo- or thermochromic system^[2]

The transformations involve the transition from a colorless to a colored state, rather than the interconversion between two colored forms. Most common photochromic molecules have a colorless or pale yellow form A and a colored form B. However, photochromic transformations are always accompanied by absorbance changes in the visible region. Thus,

visible absorption spectroscopy is the most convenient analytical method to study photochromic processes. After the photoinduced absorbance change the photochromic compound B may fade thermally back to its original state A when the irradiation is stopped. Thermally stable photochromic molecules will remain in their photogenerated state B after the irradiation is terminated.^[3] In both cases, returning to their initial state A can be achieved by decolorizing (bleaching) using visible irradiation, or generally, using irradiation at a different wavelength.

1.1.2. Photochromism in nature

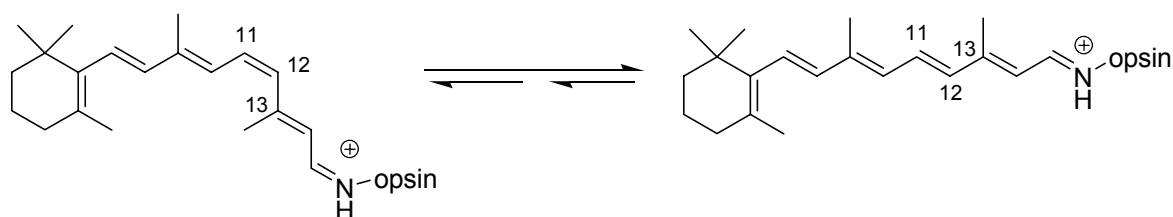
Important biological processes are triggered by light signals and controlled by the nature of the light source (i.e., wavelength and incident flux). The common feature of all these systems is the participation of chromophores (photosensor), which upon light absorption, trigger-on (switch on) a series of chemical transformations that are recognized by the surrounding biomembrane or protein assemblies. In response to the light-induced chemical transformations of the chromophore, secondary biotransformations are then initiated and complex biological events, such as neural responses or ion pumps are activated. Various biological levels, such as movement of motile organisms or intracellular movement, reveal macroscopic translocations that are initiated by light-triggered processes.^[4]

The process of vision is one of the most powerful and remarkable examples for a light-triggered complex mechanism. It involves concatenation of chemical transformations to transduce visual information into a nerve impulse using a naturally optimized, biological photoswitch.^[5] It is essential for humans and animals to recognize the outer information with their visual system, where it is processed both in the retina and in the brain. In the retina, optical inputs reach visual pigments and in the following lead to a photo-induced isomerization of retinal.^[6]



Figure 1.2. Crystal structure of bovine rhodopsin (from: PDB, File: 1L9H)

The chromophore 11-*cis*-retinal is bound via a protonated Schiff base to the protein opsin. Together they form the holoprotein rhodopsin, the human retina visual pigment. The absorption of photons results in the conversion of 11-*cis* retinal to 11-*all-trans* retinal.^[7]



Scheme 1.1. 11-*cis*-retinal to 11-*trans* retinal isomerization

This photoswitching changes the molecular shape and releases all-*trans*-retinal, which leads to further transformations that produce a sequence of chemical signals.^[5a] The *cis-trans* isomerization interacts with different protein structures, and induced conformational changes in the protein subsequently switch on amplified subsequent biochemical transformations. As a result, the initial optical information from the environment is converted by a biological organic photochromic system into an electrical signal (nerve pulse) that is transmitted to the brain by the optical nerve where it is further processed.^[6]

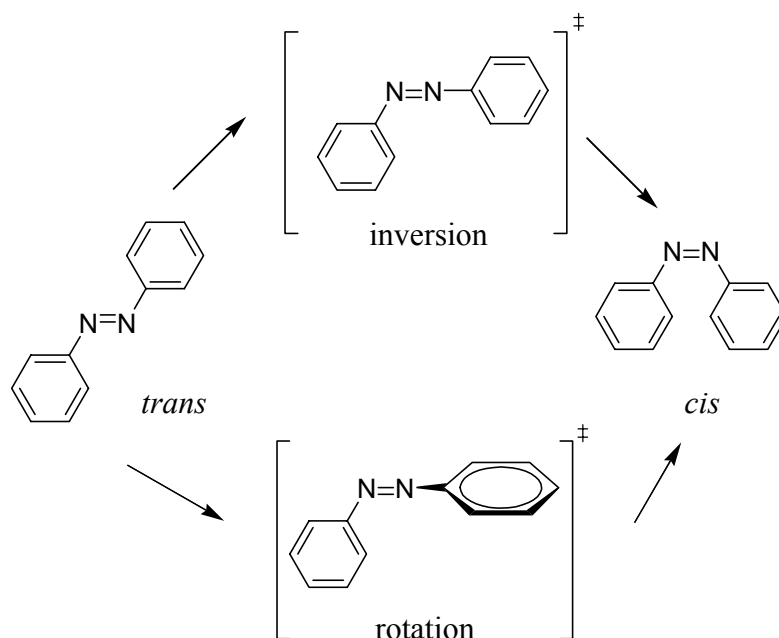
1.1.3. Organic photochromic systems

Representative examples of photochromic systems are based on transformations that are generally unimolecular or bimolecular reactions. Unimolecular photochromic processes involve the interconversion of two isomers, A to B, and can be based on photoinduced ring opening/closing,^[8] *cis-trans* isomerizations^[9] or intramolecular proton transfer.^[10] On the

other hand, bimolecular photochromic processes are less common than unimolecular photochromic processes and rely either on the photoinduced cycloaddition of two identical reactants into a single product^[11] or on the photoinduced transfer of an electron from a donor to a complementary acceptor.^[12]

1.1.3.1. *Cis-trans* isomerizations in Stilbenes and Azobenzenes

As it was already shown before, the basic process of vision is based on the *cis-trans* isomerization of retinal. The isomerization involves a 180° rotation around a carbon-carbon double bond, inducing conformational changes and eventually creating a nerve pulse for visual perception. Based on *cis-trans* isomerizations, stilbene derivatives have been studied for thermally irreversible optical switching systems.^[13]

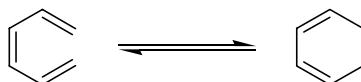


Scheme 1.2. Transition states involved in *cis-trans* photoisomerization in azobenzene^[4]

Similar to stilbene derivatives, azobenzenes are prototypes of *cis-trans* photochromes. Azobenzenes can exist in two isomeric forms, E (*trans*) and Z (*cis*) form, which display a difference in UV-absorption spectra. Due to their relatively easy synthetic accessibility and chemical robustness, azobenzenes^[14] and other *cis-trans* photochromes^[15] are widely used photochromic compounds in chemical biology.

1.1.3.2. Photocyclizations

Photochromic valence tautomerism is defined as a reversible change in color due to a shift in the position of bonds in a molecule. There, the photochemical and thermal ring opening and closing are governed by the Woodward-Hoffmann rules.^[16]

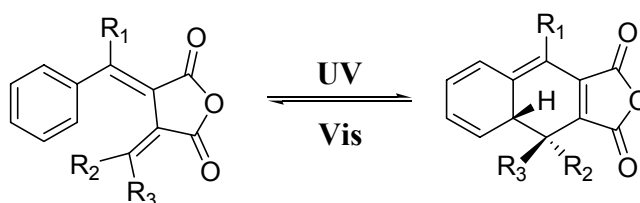


Scheme 1.3. Isomerization of 1,3,5-hexatriene and 1,3-cyclohexadiene

Photochromic systems include fulgides^[17], diarylethenes^[18] and azulen^[19]. In spirobenzopyrans and spiroxazines, the photochemical reversibility is based on heterolytic bond cleavage and a cycloreversion process.^[20]

Fulgides

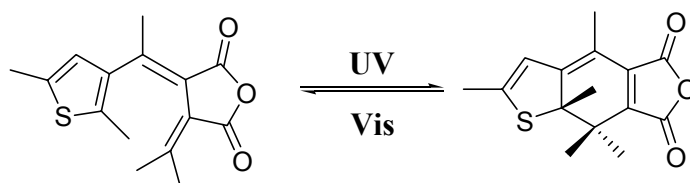
Fulgides are derivatives of dimethylenesuccinic anhydrides that are substituted with an aromatic ring. Literally, fulgides (lat.: *fulgere* = to glisten) were isolated as fine glittering crystals.^[1] The switching process in fulgides is based on a reversible photochemical conrotatory electrocyclization, similar to the 1,3,5-hexatriene cyclization. When irradiated with UV light, the mostly colorless fulgide is transformed into a colored dihydronaphthalene. The reverse reaction can be induced by irradiation with visible light.^[21]



Scheme 1.4. Fulgide to dihydronaphthalene transformation

Unwanted side reactions in fulgides are the photochemical isomerization of the *Z*-isomer to the *E*-isomer, sigmatropic proton shifts leading to undesirable side products (the hydrogen drawn in the dihydronaphthalene structure can shift to other positions) and disrotatory ring opening due to the competing fast thermal reverse reaction. Structural improvements such as the replacement of hydrogen atoms by alkyl groups can suppress the sigmatropic shift and the

thermal reverse reaction can be slowed down to a large extent by the introduction of aromatic heterocycles like furyl and thienyl groups.^[22]

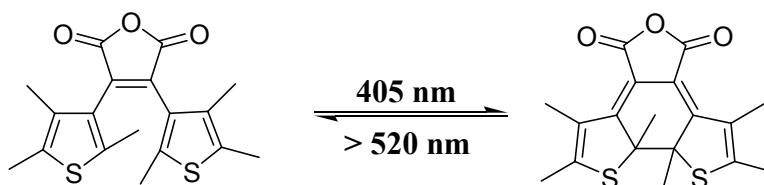


Scheme 1.5. Structurally improved fulgide

Introduction of a methyl substituent R_4 into the 2-position of the 3-thienyl group of the fulgide does not only eliminate the irreversible photochemical and thermal hydrogen-shifts, but also prevents the thermal disrotatory ring opening because of steric interactions between the R_3 and R_4 methyl groups that would arise. Conrotatory ring opening back to the E-fulgide remains unaffected. For example, the dihydrobenzo[*b*]furan is thermally stable up to 160 °C, and was converted back into the E-fulgide quantitatively upon exposure to visible light.^[23]

Diarylethenes

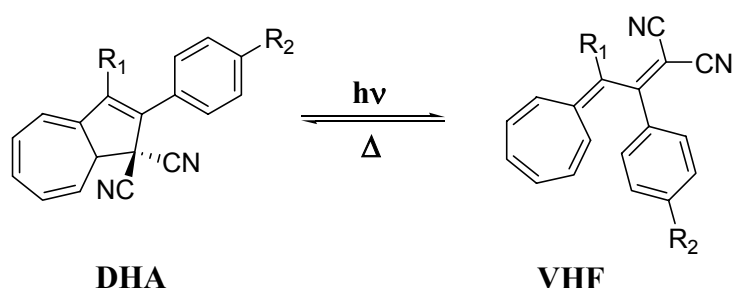
In addition to *cis-trans* isomerization, stilbene derivatives can undergo a reversible cyclization reaction upon UV irradiation. The formation of the unwanted phenanthrene derivative can be excluded by the substitution of methyl groups from the two hydrogens that are sensitive to oxidative elimination.^[24] The most commonly used diarylethenes are diarylperfluorocyclopentenes. Thermally stable diarylethenes use furan or thiophene groups instead of phenyl rings and the photochromism is based on a six-electron rearrangement.^[18]



Scheme 1.6. Example of a diarylethene photoswitch

Azulesnes

Photochromism in azulene derivatives is based on a 10-electron cycloreversion. The reversible switching of a dihydroazulene (DHA) into a vinylheptafulvene (VHF) by irradiation has been described.^[25]

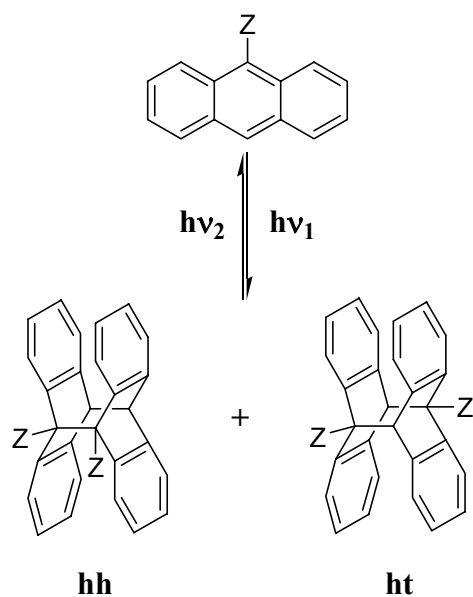


Scheme 1.7. DHA-VHF cycloreversion

The rearrangement could be rendered visibly by a change of color from yellow to red as a result from the shift of the absorption band at 350-360 nm to 460-490 nm. The VHF reverts thermally back to the DHA chromophore within seconds to days, depending upon the substitution patterns and temperature.^[19]

Anthracene

Over the last seventy years, a number of polycyclic aromatic hydrocarbons (PAH) have become infamous as biological pollutants and contaminants.^[26] Therefore, research has been stimulated, especially in the field of development of analytical methods to detect traces, which often use the efficient light emission properties of PAH.^[27] Among PAH, anthracene and its derivatives were extensively studied. Other than the before mentioned switches, anthracene and its derivatives represent bimolecular photochromic systems. Upon UV irradiation ($\lambda_{\text{max}} = 366$ nm) a [4+4]-cycloaddition switches the anthracene monomer into its dimer.^[28] The photochromic properties of anthracenes are of special interest in the field of optical and electronic switches.^[11]

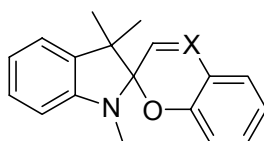


Scheme 1.8. Photodimerization of 9-substituted anthracene derivative. Head-to head (hh) and head-to-tail (ht) photodimers (from Lit ^[11])

Interestingly, the two monomers can associate with a head-to-head (hh) or a head-to-tail (ht) mutual orientation, leading to the hh and ht photodimers, but steric hindrance can lead to the preferential ht oriented dimers. From the anthracene-dimer, the back reaction can then be induced by light or by temperature. Also, intramolecular photodimerization of anthracene has been used for molecular or ionic receptors^[29] and for the design of binary optical memory.^[30]

Spirobenzopyrans and Spiroxazines

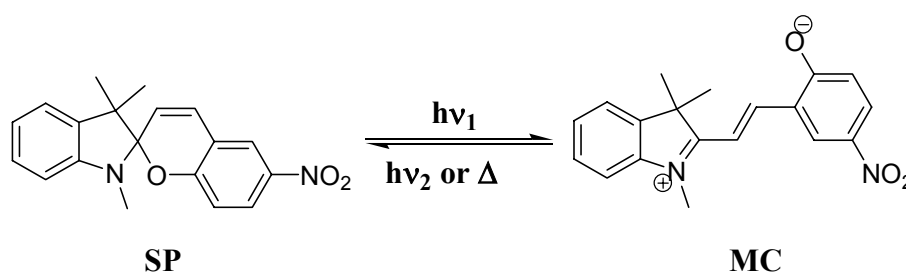
Spirobenzopyrans (also: spiropyrans) and spiroxazines (also: spirooxazines) belong to the very large group of photochromic switches that are based on ring opening and ring closing reactions by photo-induced electrocyclic reactions. Spirobenzopyrans consist of conjugated rings and a pyran fragment. The pyran moiety itself usually belongs to a larger aromatic system, whereas the other heterocyclic part is often based upon mono or bi-heteroatomic azaheterocycles. The two heterocyclic parts are linked together by a common spiro carbon atom. This aligns the two halves of the molecule in two orthogonal planes.^[31]



Scheme 1.9. General structure of spiropyrans (X = CH) and spiroxazines (X = N)

Photochromism of spirobenzopyrans has first been investigated in 1952 by Hirshberg and Fischer. Based on their photoswitching properties, research has been carried out with spirobenzopyrans for applications as molecular logic elements^[32], light-actuated nanovalves^[33] and manipulation of in vitro selection processes by light-regulation.^[34] Hirshberg already suggested an idea of using the photoswitching process as basis for photochemical erasable memory devices in his early work^[35]. More than 30 years later the use of spirobenzopyrans has been demonstrated for bit-oriented three-dimensional optical data memory systems.^[36]

The photochromic and thermochromic behaviour of spirobenzopyrans is due to the interconversion between the closed form SP and the open merocyanine dye MC. In the closed SP form, the spirobenzopyrans usually have an absorption maximum in the UV range. UV irradiation then leads to the open MC, which can revert by irradiation with visible light and thermally to the closed SP.



Scheme 1.10. Interconversion of SP and transoid MC form

Upon UV irradiation, the C-O bond in the spirobenzopyran form (SP) is heterolytically cleaved. The cleavage then allows the molecule to unfold and the ring-opening leads to structural changes facilitating the photomerocyanine form (MC).^[20] The bond cleavage between the spiro carbon and oxygen is assumed to lead to the formation of primary photoproducts, with orthogonal parent geometry, but different stereoisomers of cisoid configuration. This is followed by a geometrical change to form a planar transoid, isomerized configuration of merocyanine. The transition itself, from SP to the zwitterionic MC form occurs on a picosecond to nanosecond time-scale.^[17] The isolated systems, which are orthogonal and non-interacting in the SP form, and show no absorption in the visible spectrum, become extensively conjugated in the colored MC form.

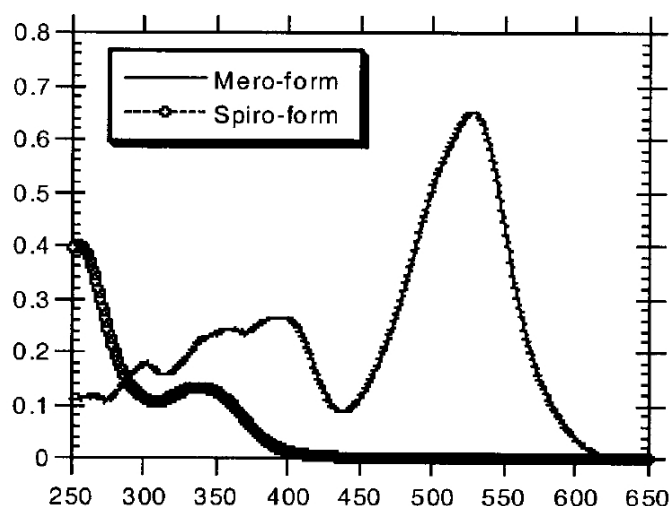
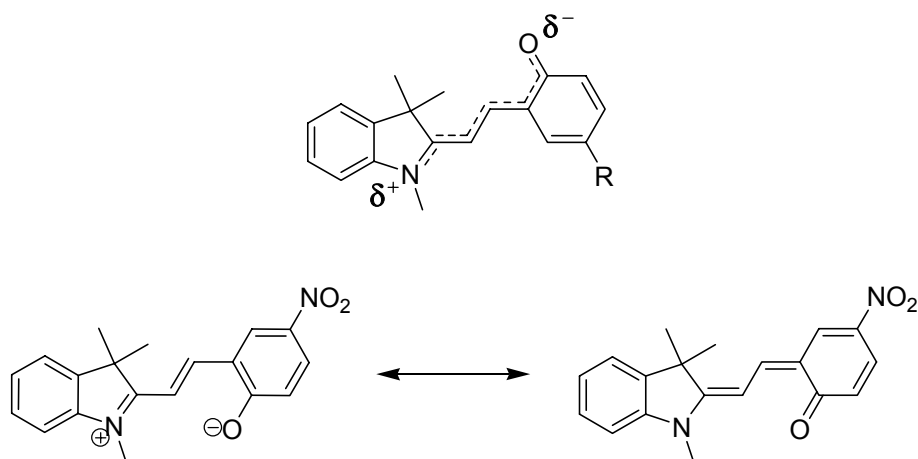


Figure 1.3. Absorption spectra for a spirobenzopyran in MeCN^[37]

The zwitterionic MC form shows a strong and characteristic absorption band in the visible wavelength range due to its extended conjugation of the π -electron system as compared to the unfolded SP form with orthogonal geometry.^[38]

The electronic distribution in the photoproduct, the transoid merocyanine, can be symbolized by repartition of the delocalized π -electrons with an excess of positive charge on the heterocycle, and an excess of negative charge on the phenolic oxygen.

Corresponding to the experimental situation, the dipolar zwitterionic merocyanine form with localized charges and the nonionic quinoid form may have important contributions. This already shows that the photochromic equilibrium is more complex, and for complete understanding it would be necessary to also take the different geometries and electronic structures into account.^[31]



Scheme 1.11. Charge distribution of the colored MC form and two possible states for zwitterionic and apolar quinoid MC

However, the zwitterionic MC form can be stabilized in polar solvents, which lead to a larger energy of activation and therefore to a slower back reaction to the closed SP form. The rearrangement from open MC to closed SP form in a back reaction can occur, since the carbon-oxygen cleavage is photochemically and/or thermally reversible. Hence, the colored MC can revert back to SP either via thermal process or a second photochemical step that is triggered by absorption of light at the wavelength of λ_{max} of the colored form of the MC.^[3, 20]

The spiro carbon atom itself is a stereogenic center in the spirobenzopyrans. But as a consequence of the achiral nature of the merocyanine form, the photochromic process always leads to racemization.^[39] However, when a chiral substituent remote from the spiro center is present, diastereoisomers of spiropyranes can also be isolated.^[40]

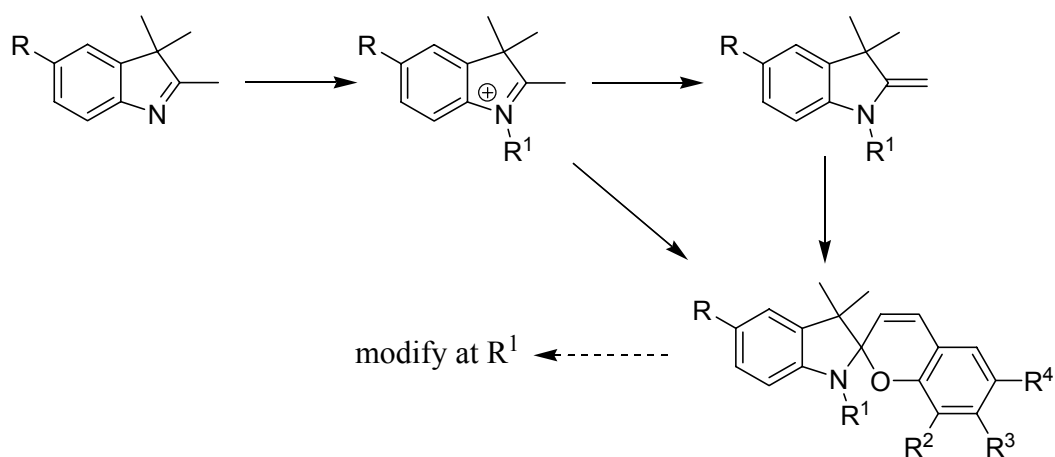
Similar to spirobenzopyrans, the photochromic properties of spiroxazines are based on the cleavage and reformation of the carbon oxygen bond of the oxazine ring. The ring-opened merocyanine absorbs in the region of 600 nm and is recognized by the blue color. Spiroxazines have excellent resistance to light-induced degradation and show a high number of photoswitching cycles.^[41] Thus, the vast majority on spiroxazines are published in patents and patent applications. They have been successfully applied, for example in optical filters, lenses and eyewear.^[42] Applications of photochromic systems such as spirobenzopyrans, azobenzenes and other photo-responsive molecules in chemical biology and nanotechnology are discussed in Chapter 3.

In order to endow biomolecules with the photochromic switch, a versatile and improved synthetic route for spirobenzopyrans that are fitted up with iodo, hydroxyl, azido, ethynyl or carboxylic acid groups is reported. These spirobenzopyrans represent important building blocks that can be used for cross-linking or bioconjugation reactions to attach the photochromic compound to biopolymers or functional π -systems.

1.2. Results and Discussion

Our synthetic strategy was to synthesize the functionalized spiroindolinobenzopyran switches by using the following assemblies:

- N-alkylation of indolines with a linear C3-linker bearing a hydroxyl or iodo group (R^1), followed by alkali treatment and condensation with salicylaldehyde derivatives; further transformations should be carried out at the C3-linker (R^1)
- Preparation of functionalized salicylaldehydes (R^2 , R^3 , R^4) and condensation with Fischer's base or the corresponding quaternary salt
- Modification at the indoline section (R) and formation of a spirobenzopyran



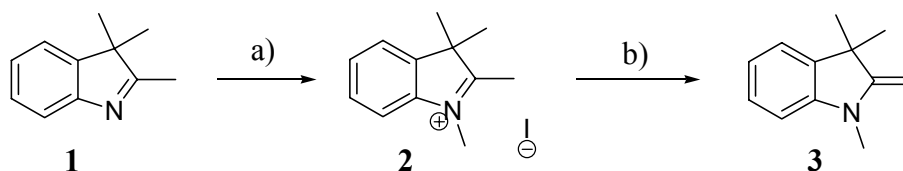
Scheme 2.1. Route for functionalization of spirobenzopyran switches

In general, Fischer's base or other N-alkylated indolenines are obtained from their corresponding quaternary salt by deprotonation at the C2-methyl group. The indolenines can be isolated or generated in situ and then condensation with salicylaldehyde derivatives is usually carried out in dry ethanol under reflux. The nucleophilic attack of Fischer's base or an

indolenine at the carbonyl group forms an aldol product, followed by dehydration and ring-closure. Detailed mechanisms are reported in the literature.^[43]

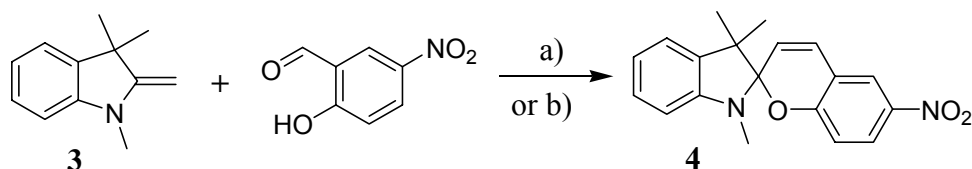
1.2.1. Synthesis of N-methylated spiroindolinobenzopyran

The synthesis of spirobenzopyrans started with the N-alkylation of 2,3,3-trimethylindolenine **1** with iodomethane under reflux in acetonitrile for 24 hours and afforded the indolium iodide **2** as fuzzy powder in 74 % yield. Interestingly, synthesis of **2** was also performed using conditions from the literature.^[44] Therefore, the corresponding starting materials were stirred at room temperature in nitromethane for 12 hours. Unlike the excellent yield reported in the literature (96 %), the desired product could not be isolated in any satisfactory amounts. In the second step, the indolium salt **2** was deprotonated at the C2-methyl position with use of potassium hydroxide. The product **3** (Fischer's base) was easily extracted from the reaction mixture with use of diethyl ether, and the organic layer turned pink within few minutes. A red oil was obtained after the solvents were evaporated in vacuo. Although Fischer's base is commercially available, distillation prior to use was crucial. Experiments for the preparation of spirobenzopyrans using "red" Fischers's base always gave black-brown products that required multiple recrystallizations in ethyl acetate with charcoal, and gave the desired products only in low yields. However, after deprotonation of **2** with potassium hydroxide and distillation, **3** was afforded as colorless oil in 77 % yield (Scheme 2.2).



Scheme 2.2. Synthesis of **3**. Reagents and conditions: a) CH₃I (1.1 eq.), MeCN, reflux, 24 h, 74 %; b) KOH (3.3 eq.), r.t., 3 h, 77 %.

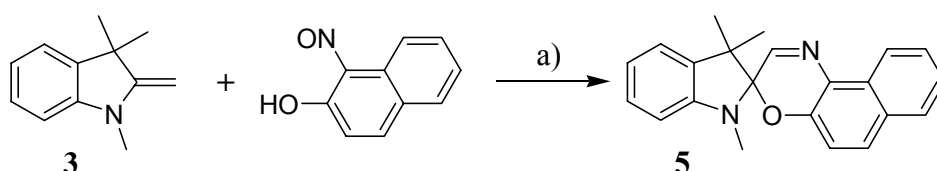
Equimolar amounts of 2-hydroxy-5-nitrobenzaldehyde and **3** were then reacted, either under reflux or ultrasonic irradiation^[45], to give the N-methylated spirobenzopyran **4**. Reaction control by TLC revealed that ultrasonic irradiation decreased the reaction time and both reaction setups gave the desired product **4** in excellent yield (Scheme 2.3).



Scheme 2.3. Synthesis of **4**. Reagents and conditions: 2-hydroxy-5-nitrobenzaldehyde (1.0 eq.); a) EtOH, reflux, 4 h, 97 %; b) EtOH, 80 min, 94 %.

1.2.2. Synthesis of N-methylated spiroxazine

Spiroxazines are aza analogs of spirobenzopyrans since the CH at the 3-position is replaced by a nitrogen atom. Spironaphthoxazines are generally prepared similar to spirobenzopyrans but with 1-nitroso-2-naphthol derivatives instead of salicylaldehydes, also in methanol or ethanol under reflux.^[46] We investigated the use of ultrasonic irradiation as an alternative to reflux conditions for the preparation of an N-methylated spiroxazine **5**. Therefore, equal amounts of **3** and 1-nitroso-2-naphthol were subjected to ultrasonic irradiation in MeOH. The reaction was followed by TLC and appearance of a green-brown spot clearly indicated formation of the photochromic spiroxazine **5**. After 2 hours the reaction was stopped and **5** was obtained after flash chromatography in 65 % yield as lustrous orange solid (Scheme 2.4). Comparison with yields reported in literature (reflux^[47]: 47 %, microwave^[48]: 67 %) show that ultrasonic irradiation can be used as a suitable method for the preparation of **5**.

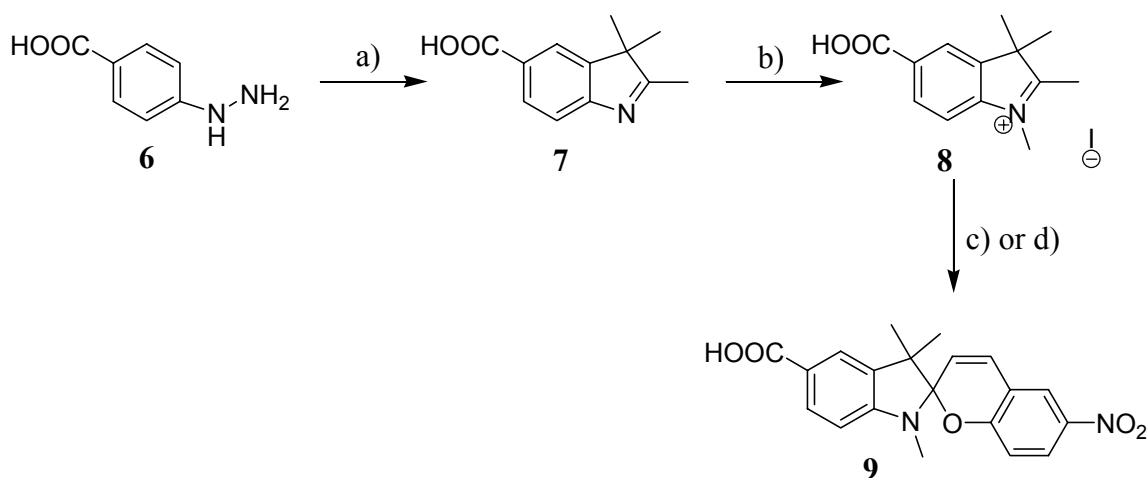


Scheme 2.4. Synthesis of **5**. Reagents and conditions: a) MeOH, 2 h, 65 %.

1.2.3 Synthesis and photoswitching of N-methylated spirobenzopyran carboxylic acid

Functional molecules with carboxylic groups play a major role in biotechnological applications, especially as precursors for labeling procedures.^[49] Formation of stable amide bonds in labeling applications can be performed by using NHS-esters, with carboxylic acids as precursors. With use of NHS and an activating agent like DCC or EDC, the synthesized activated NHS-esters, i.e. the photochromic switches will react readily with amino functions of various biomolecules.^[50] The synthetic approach for the potentially useful building block **9** involved three steps. First, **7** was prepared in a Fischer indole synthesis. Therefore,

commercially available 4-hydrazinylbenzoic acid **6** was reacted with 3-methyl-2-butanone in ethanol with sulfuric acid to give **7** as orange-yellow solid in 63 %. N-Alkylation of **7** with an excess of iodomethane under reflux in chloroform gave the corresponding carboxy-indolium iodide **8** in 81 % yield. For the preparation of **9**, 2-hydroxy-5-nitrobenzaldehyde and a slight excess of piperidine was then reacted with **8** under reflux for 18 hours.^[50] Upon cooling, the product precipitated and recrystallization from ethanol afforded the desired product (94 %). However, the last reaction step for the formation of **9** was also performed under ultrasonic conditions and gave **9** already after 60 minutes irradiation in nearly quantitative yield as orange-green crystals (Scheme 2.5).



Scheme 2.5. Synthesis of **9**. Reagents and conditions: a) 3-methyl-2-butanone (1.1 eq.), H₂SO₄, EtOH, reflux, 16 h, 63 %; b) CH₃I (2.1 eq.), CHCl₃, reflux, 18 h, 81 %; 2-hydroxy-5-nitrobenzaldehyde, (1.0 eq.) c) piperidine (1.0 eq.), reflux, 18 h, 94 %, d) piperidine (1.2 eq.),)), 60 min, 98 %.

The irradiation of a solution of **9** in ethanol visualizes the photochromic switching (Figure 2.1). First, the solution was bleached with an amber LED ($\lambda = 590$ nm) to switch **9** to its SP form. Then, the sample was irradiated with UV light ($\lambda = 312$ nm) for 30 seconds and an absorption spectrum was recorded (MC 1). This UV irradiation was iterated three times (MC 2-MC 4).

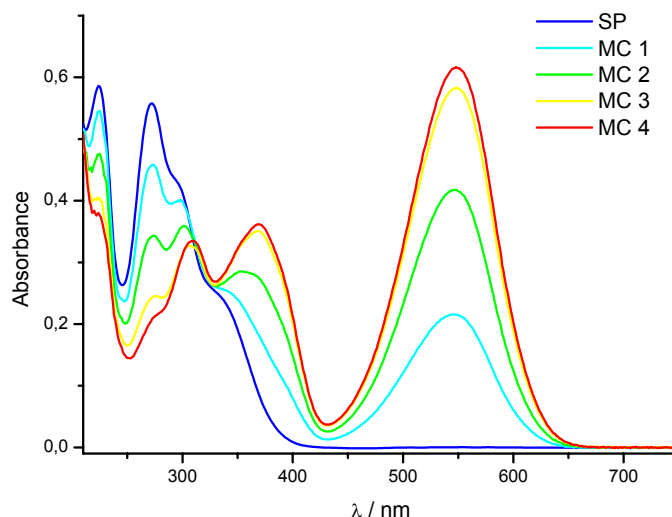


Figure 2.1. Absorption spectra of **9** in ethanol ($c = 100 \mu\text{M}$)

Irradiation with 312 nm leads to a color change of the solution, which turns from colorless to red-pink. Here, the colorless SP form of **9** is switched into its corresponding photomerocyanine state. The inspection of the absorption maximum ($\lambda_{\text{max}} = 549 \text{ nm}$) over time displays the achievement of a photostationary state under the employed irradiation conditions after 2 minutes (Figure 2.). The photoswitching process can also be monitored by differential absorption spectra. The inset shows the decrease of absorption bands in the UV range at 224 nm and 272 nm, while two new significant absorption bands arise at 369 nm and 549 nm that can be attributed to the open merocyanine form.

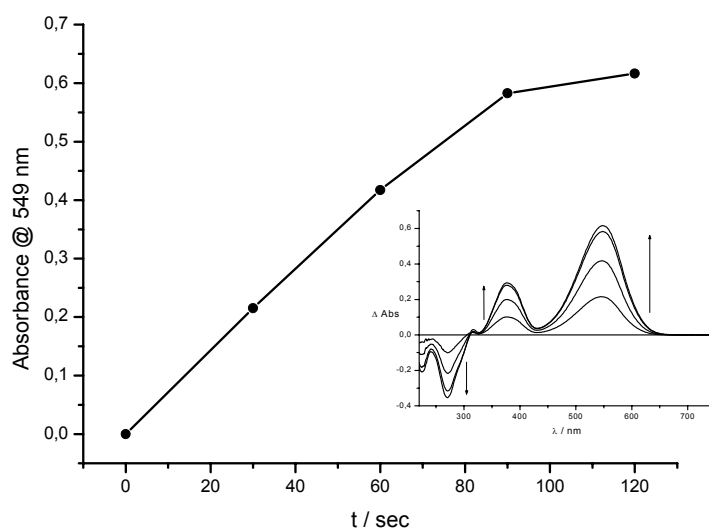
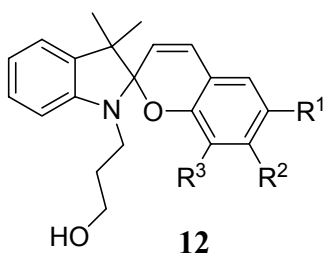


Figure 2.2. Increase of λ_{max} during irradiation with $\lambda = 312 \text{ nm}$

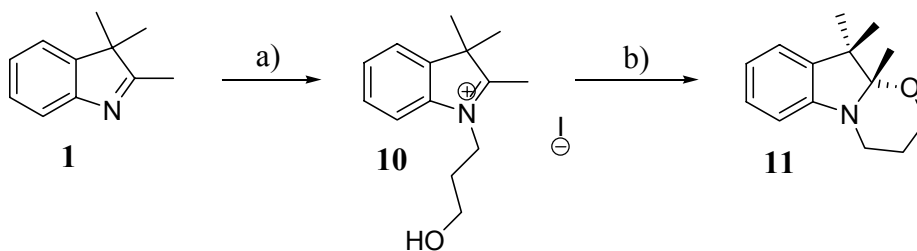
1.2.4. Synthesis and optical properties of spirobenzopyrans with a linear C3-linker with hydroxyl group

Spirobenzopyrans with the general structure **12** were prepared (Scheme 2.6). They are all decorated with a propanol-chain at the indole-N, bear two methyl groups at the 3-position at the indole-half and are distinguished by variation of substituent groups and positions in the benzopyran section.



Scheme 2.6. General structure of spirobenzopyran **12** with substituent variations R^1 , R^2 , R^3

As depicted in Scheme 2.6, the N-alkylation of **1** with 3-iodopropanol in chloroform under reflux for 24 hours gave in very good yield the corresponding indolium iodide **10** that carries the propanol-linker. Deprotonation of **10** with potassium hydroxide provided the stable tricyclic oxazino indole **11** after flash chromatography in 43 % yield. **11** was characterized by 2D NMR and structural evidence was also confirmed by X-ray analysis, after we were able to grow colorless crystals of **11** from a concentrated solution in ethyl acetate.



Scheme 2.7. Synthesis of **11**. Reagents and conditions: a) $I(CH_2)_3OH$ (1.1 eq.), $CHCl_3$, reflux, 24 h, 99 %; b) KOH (2.4 eq.), r.t., 2 h, 43 %.

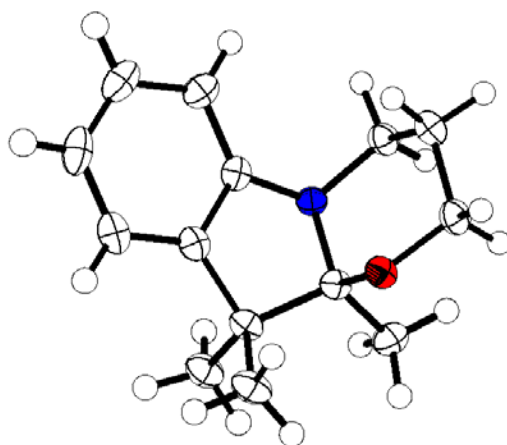
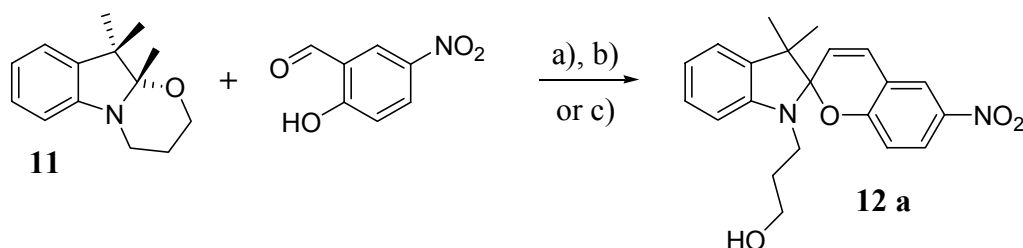


Figure 2.3. X-ray crystal structure of compound **11**.

The spirobenzopyran **12 a** (Scheme 2.6, $R^1 = \text{NO}_2$, $R^2, R^3 = \text{H}$) was synthesized, according to the most common protocol. Thus, 2-hydroxy-5-nitrobenzaldehyde and **11** were refluxed in ethanol. First, a synthetic protocol comparable to the literature was performed. Therein, the related compound, bearing an ethanol-linker, instead of a propanol-linker, is synthesized by refluxing the corresponding starting materials in ethanol for 3 hours and obtained as a purple solid in 81 % yield.^[51] However, our synthetic approach only gave **12 a** in a poor yield of 16 %. Prolonged heating was also tested (24 hours reflux), and increased the yield to 54 %. However, heating for more than 24 hours did not increase the yield further, but increased the formation of side-products that complicated further purification. A reasonable synthetic method to increase the yield of the reaction was the use of ultrasonic irradiation, since it is known to be a valuable tool for the performance of aldol-condensations.^[45, 52] We used equimolar amounts of **11** and 2-hydroxy-5-nitrobenzaldehyde, and exposed this mixture in ethanol to ultrasonic irradiation. The formation of the photochromic product was already detectable after few minutes. TLC was used to monitor the performance of the reaction and after 1 hour the product spot intensity was steady in successive controls. Then, standard workup and purification by flash chromatography provided the desired product **12 a** with an increased yield of 84 % (Scheme 2.8).



Scheme 2.8. Synthesis of **12 a**. Reagents and conditions: a) EtOH, reflux, 3 h, 16 %; b) EtOH, 24 h, 54 %; c) EtOH, 1 h, 84 %.

Unlike the before mentioned N-methylated spirobenzopyrans, those with the general structure **12** afforded special attention during workup. The existence of the C3-linker with the hydroxyl group showed that these spirobenzopyrans could not be recrystallized as nicely as the N-methylated compounds, even in solvent mixtures.^[53]

However, purification employing flash chromatography was possible, but we had to act with caution running the column.^[54] If the crude products were applied as a dry-load the desired product stuck to the silica gel and would only elute upon addition of ammonium acetate to the mobile phase. The usually blood-red color of the dry-load (and eluting sections) showed that the very polar silica gel led to an increase of the colored, zwitterionic merocyanine form and therefore to a mixture of the two forms that would smear all over the column. Concurrently, the use of ammonium acetate also led to a broadening of the eluting band. When using dry-vacuum flash chromatography we observed identical problems as with dry-loading.^[53a, 55] Likewise, preparative thin layer chromatography has also been performed. In the dark, the method showed promising results for purification since the retention factors are directly comparable to the ones obtained through TLC. Handling of the plates in the dark and a limited capacity of ~ 50 mg crude product load per plate for best purification results led us to optimize flash chromatography, but without use of ammonium acetate.^[53a] The fact that the desired photochromic compounds can consist of a mixture of isomeric spirobenzopyrans and merocyanines also signify the difficulty in handling.

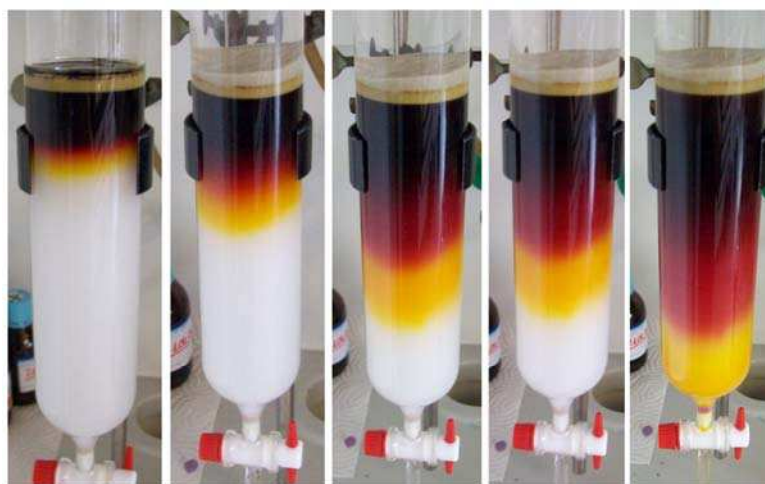
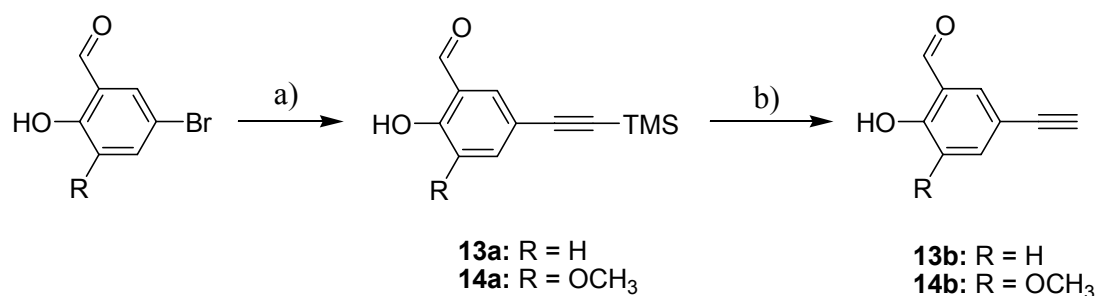


Image 2.1. Visual representation of isomerization of a typical spirobenzopyran **12** during flash chromatography under normal laboratory ambient light

Image 2.1 shows the isomerization during flash chromatography. For illustrative purpose of the challenges during separations, an already purified spirobenzopyran of general structure **12** has been applied representatively. Under dark room conditions, the smooth transition between the yellow band and the red band vanishes (i.e., the yellow band gets more intense and sharper). Variation of product loading methods and eluent gradient mixtures, overall remaining time on the column and change of incident light showed that pure spirobenzopyrans of general structure **12** could be isolated with a high recovery after chromatography, when the crude product was loaded in as little dichloromethane, hexane or heptane as possible and not as dry-load. All further steps were also carried out in the absence of diffuse or direct daylight (i.e., at least darkroom or better, dark conditions), eluting the desired spirobenzopyran in less than 45 minutes to prevent isomerization to the more polar merocyanines. Drying of the solvents that are used as the mobile phase and very affectionate increase of solvent polarity during gradient elution also contributed to major improvements. As a result of these wayside findings, we applied this general strategy also for purification of the following spirobenzopyran derivatives, whenever recrystallization did not afford the pure products.

To expand the range of substituents at the salicylaldehydes used for the formation of spirobenzopyran compounds **12**, two ethynyl-salicylaldehydes were synthesized.^[56] Since terminal alkynes have caused a stir with respect to “click” reactions, ethynyl-modified spirobenzopyrans constitute reasonable building blocks.^[57] The methoxy substituent also has the potential to expand photochromic spirobenzopyrans to form complexes with certain metal ions in solution in their respective merocyanine form.^[58]

The synthesis started from 5-bromosalicylaldehyde (or its 3-methoxy derivative, respectively) with a Sonogashira cross-coupling reaction with little excess of ethynyltrimethylsilane, while copper iodide and $\text{PdCl}_2(\text{PPh}_3)_2$ were used as catalysts. The TMS-protected products **13 a** and **14 a** were obtained in good yields and consequent desilylation went smoothly by using a freshly prepared solution of Bu_4NF in tetrahydrofuran to provide **13 b** and **14 b** as solids in excellent yields (Scheme 2.9).



Scheme 2.9. Preparation of **13 b** and **14 b**. Reagents and conditions: a) R = H: $(\text{CH}_3)_3\text{SiCCH}$ (1.6 eq.), $\text{PdCl}_2(\text{PPh}_3)_2$ (3.2 mol-%), CuI (3.5 mol-%), NEt_3 , 80 °C, 3 h, 88 %; R = OCH₃: $(\text{CH}_3)_3\text{SiCCH}$ (1.6 eq.), $\text{PdCl}_2(\text{PPh}_3)_2$ (3.4 mol-%), CuI (3.9 mol-%), NEt_3 , 80 °C, 3 h, 73 %; b) R = H: Bu_4NF (1.9 eq.), THF, r.t., 30 min, 92 %; R = OCH₃: Bu_4NF (1.9 eq.), THF, r.t., 30 min, 94 %.

With reasonable quantities of **11** and differently substituted salicylaldehydes in hand, the previously described protocol for **12 a** using ultrasonic irradiation was applied to the synthesis of other photochromic spirobenzopyrans and results are listed below (Table 2.1).

Compound	R ¹	R ²	R ³	Yield (%)
12 a	NO ₂	H	H	84
12 b	H	H	H	87
12 c	Cl	H	H	78
12 d	OCH ₃	H	H	54
12 e	Br	H	Br	93
12 f	C≡CH	H	H	68
12 g	C≡CH	H	OCH ₃	75
12 h	Br	H	H	52

12 i	NO ₂	H	OCH ₃	83
12 j	Br	H	OCH ₃	76
12 k	H	OCH ₃	H	82

Table 2.1. Substituents and yields of spirobenzopyrans **12 a** - **12 k**

As Table 2.1 shows, eleven differently substituted spirobenzopyrans, based on the general structure **12**, were obtained with the use of ultrasonic radiation. The reaction times were 1-2 h in EtOH and **12 a** - **12 k** were afforded in acceptable to excellent yields, ranging from 52-93 %. It can be assumed that, basically, ultrasound simply involves a more intimate mixing of the starting materials and therefore enhances the reaction rates. This effect has also been observed for other organic reactions.^[52]

As Table 2.2 shows, the synthesized spiropyrans **12 a** - **12 k** have different substitution patterns at their extended aromatic pyran part, including electron-withdrawing and -donating substituents.

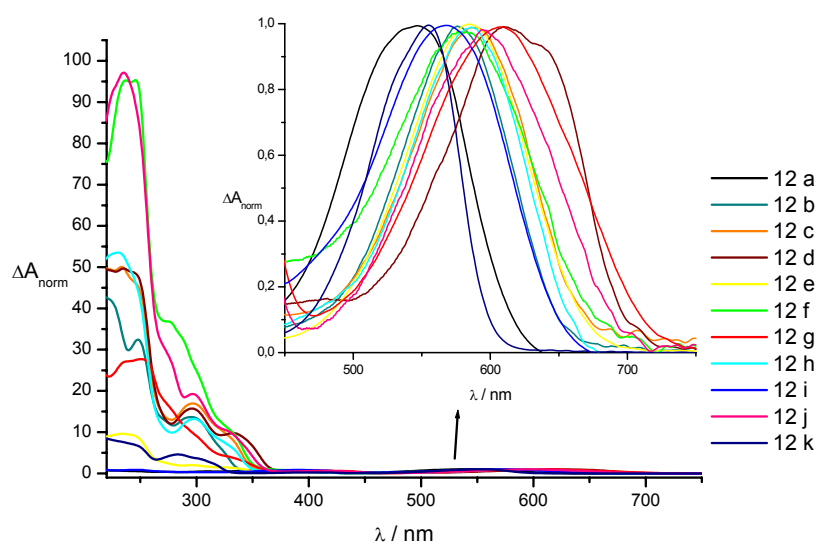


Figure 2.4. UV/Vis absorption of the merocyanines **12 a** - **12 k** (100 μ M in ethanol, after irradiation at $\lambda = 312$ nm)

Accordingly, solutions of the spiropyrans in ethanol were bleached with visible light with use of a high-power output amber LED ($\lambda = 590$ nm) until no significant change in the visible range could be detected. In the following, the samples were irradiated with UV light ($\lambda = 312$

nm) and the photocoloration properties were then measured immediately after saturation of the merocyanines.

Compound	R ¹	R ²	R ³	λ_{max}^*
12 a	NO ₂	H	H	549
12 b	H	H	H	577
12 c	Cl	H	H	592
12 d	OCH ₃	H	H	606
12 e	Br	H	Br	586
12 f	C≡CH	H	H	586
12 g	C≡CH	H	OCH ₃	606
12 h	Br	H	H	586
12 i	NO ₂	H	OCH ₃	568
12 j	Br	H	OCH ₃	599
12 k	H	OCH ₃	H	554

Table 2.2. Substituents and λ_{max} of merocyanine forms of **12 a** - **12 k**. *: 100 μM in ethanol, after irradiation at $\lambda = 312$ nm.

Spirobenzopyran **12 b** carries hydrogen atoms at the positions R¹, R² and R³ and can be taken as reference compound to depict substituent effects. **12 b** has λ_{max} of 577 nm in ethanol, and from this we can conclude some trends. First, inductively electron-withdrawing groups which are electron-donating through resonance (e.g. the Cl and Br groups in **12 c**, **12 e** and **12 h**) have a minimal bathochromic effect. The ethynyl substituent (**12 f**) also leads to a small bathochromic shift. Additional substitution with the electron-donating methoxy-substituent (**12 d**, **12 g** and **12 j**) enlarges the bathochromic shift by resonance (e.g. for **12 d** to **12 b**, $\Delta\lambda = 29$ nm). Although **12 k** has one methoxy substituent, a small hypsochromic effect is observed. This can be explained by its meta-position, relative to the O⁻ of the corresponding merocyanine form. The hypsochromic shift is more distinct with the strong electron acceptor group NO₂. Albeit spirobenzopyran **12 i** has the electron-donating methoxy group, the more distinctive electron-drawing of the nitro group leads to a hypsochromic effect. Compared to **12 b**, the spirobenzopyran **12 a** displays the strongest hypsochromic shift (for **12 a** to **12 b**, $\Delta\lambda = 28$ nm) since **12 a** features only the strong electron-withdrawing nitro group.

The different substituents did not only effect the positioning of the absorption maximum of the merocyanine form, but also the stability. Although all of the above described compounds

are photochromic and absorption spectra could be recorded for the photomerocyanine form using fast scans, most of them also reverse extremely rapid back to their spirobenzopyran form. Preferentially electron donating groups destabilize the merocyanine and reverted quickly back to their corresponding spiropyran form. On the other hand, due to their nitro groups, the photochromic spiropyrans **12 a** and **12 i** show reasonable stability and strong extinction of the merocyanine at 549 nm or 568 nm, respectively.

The reversibility of the photoinduced switching was confirmed, representatively for **12 h** as shown in Figure 2.5. Therefore, a solution of **12 h** was irradiated with visible light (1). Following, irradiation with UV light led to the formation of the colored photomerocyanine form with λ_{max} 586 nm (2). The closing to form the colorless spiropyran (3) and switching to the open merocyanine (4) was repeated photochemically.

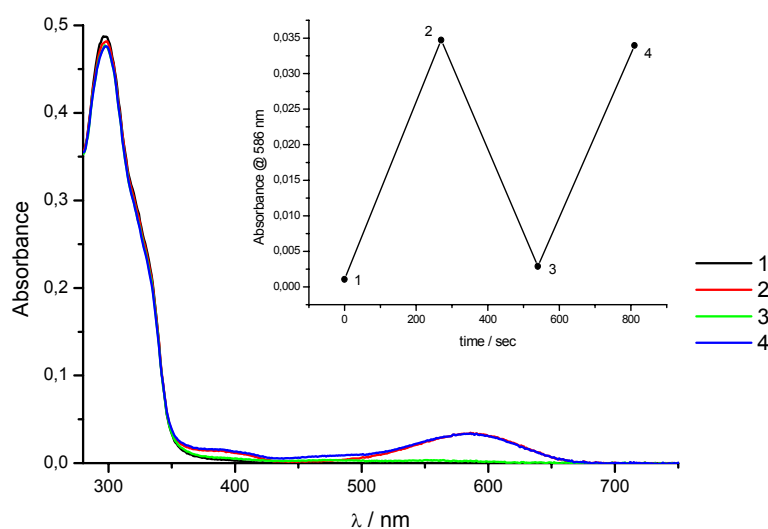


Figure 2.5. UV/Vis absorption of **12 h** (100 μM in EtOH). **1**: After irradiation with $\lambda = 590$ nm, **2**: After irradiation with $\lambda = 312$ nm for 270 sec, **3**: After irradiation with $\lambda = 590$ nm for 270 sec, **4**: After irradiation with $\lambda = 312$ nm for 270 sec

The UV induced ring-opening was also followed for **12 a** (Figure 2.6). A solution of the spirobenzopyran was first bleached with visible light (1) and then irradiated with UV light ($\lambda = 312$ nm). The inset displays the absorption change at λ_{max} 549 nm and shows that with the used irradiation setup the photostationary state is reached after 2.5 minutes.

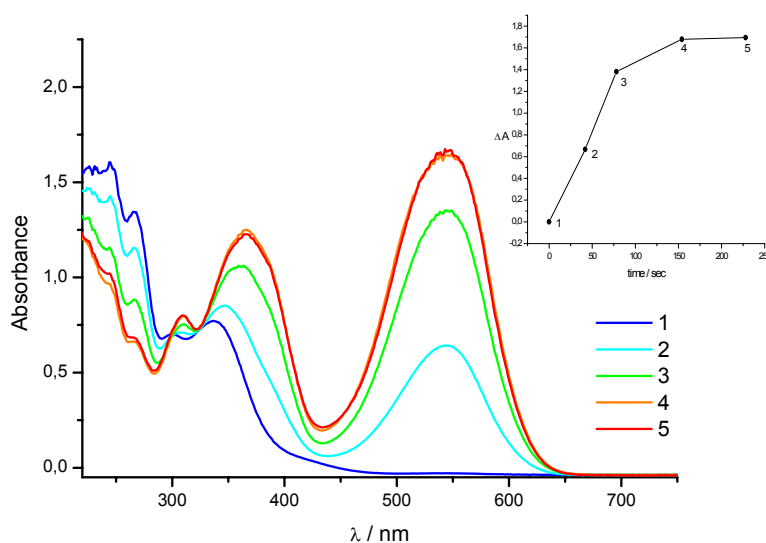


Figure 2.6. UV/Vis absorption of **12 a** (100 μM in EtOH). **1**: After irradiation with $\lambda = 590$ nm, **2**: After irradiation with $\lambda = 312$ nm for 42 sec, **3**: After irradiation with $\lambda = 312$ nm for 78 sec, **4**: After irradiation with $\lambda = 312$ nm for 154 sec, **5**: After irradiation with $\lambda = 312$ nm for 228 sec

Moreover, the photo-induced ring opening of spirobenzopyran **12 a** with different UV wavelengths was investigated and is displayed in Figure 2.7.

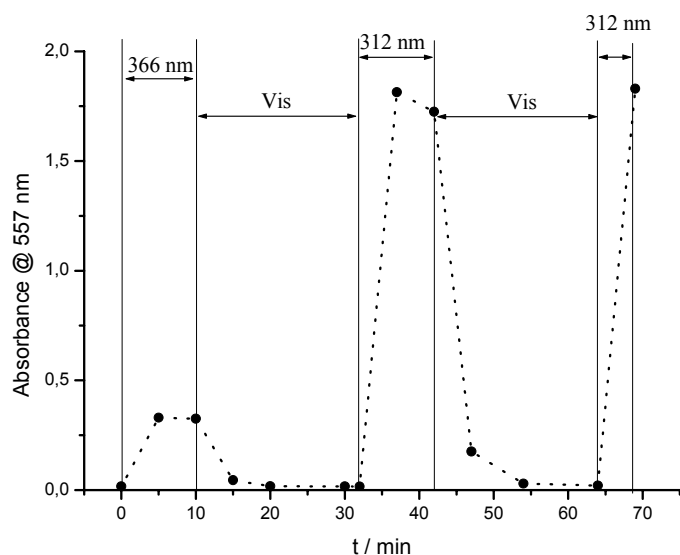


Figure 2.7. Change of λ_{max} (557 nm) of **12 a** (100 μM in MeCN) using $\lambda = 312$ and 366 nm.

A bleached solution of **12 a** in acetonitrile was irradiated with $\lambda = 366$ nm ($t = 0$) and absorption at λ_{max} (557 nm) was recorded after 5 and 10 minutes irradiation time. Then, the formed merocyanines were switched back by irradiation with visible light for 22 minutes. Respectively, the sample was then irradiated with $\lambda = 312$ nm for 5 and 10 minutes and showed a more than 5-fold increase at the absorption maximum. Bleaching with visible light for 22 minutes reveals the reversibility of the photoswitching cycle for **12 a**. Finally, the photostationary state could again be reached successfully after repeated irradiation with $\lambda = 312$ nm for 5 minutes.

Likewise, the solvatochromism of **12 a** was investigated. It is known that position, shape and intensity of UV/Vis/NIR absorption spectra may be influenced by solvents.^[59] The solvatochromic behaviour of a dye molecule (i.e. its tendency to undergo a bathochromic or hypsochromic shift with increasing polarity), depends largely on the change in dipolar characteristics between the ground and first excited states. For a weakly polar molecule, with low polarity in the ground state and increased polarity in the excited state, a bathochromic shift results, and this is termed positive solvatochromism. Conversely, for a highly polar molecule in the ground state with reduced polarity in the excited state, a hypsochromic shift is observed (negative solvatochromism).^[59-60] In principle, a merocyanine dye could exhibit either type of behaviour since the ground state could have an electronic configuration corresponding largely to the nonpolar amino-keto form or to the dipolar zwitterionic form.

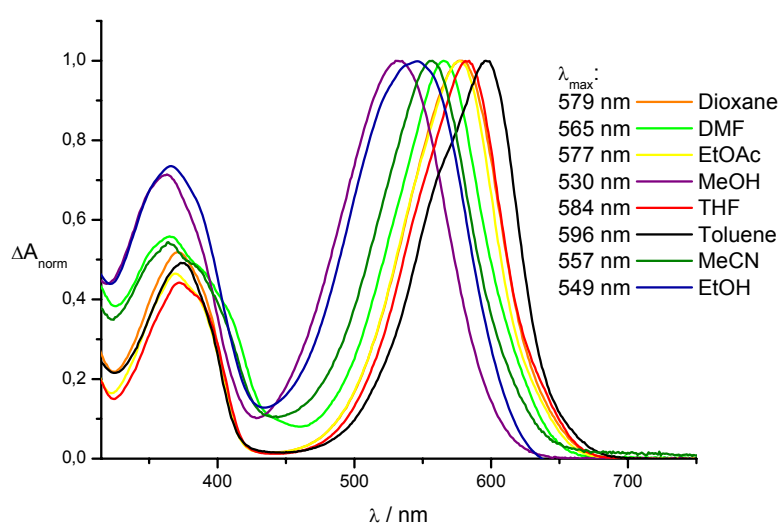


Figure 2.8. Normalized absorption spectra of **12 a** in different solvents after UV irradiation

Figure 2.8 shows the normalized absorption spectra of **12 a** in different solvents after UV irradiation ($\lambda = 312$ nm). The maximum absorption band of the merocyanine form of **12 a** undergoes a large blue-shift with increasing solvent polarity (66 nm from toluene to MeOH). As a polarity parameter, the change in transition energy has been established as the E_T value of the solvents.^[59] The reasonable linear plot of **12 a** (Figure 2.9) was obtained on plotting λ_{\max} versus the solvent parameter E_T .^[61] It clearly shows that upon polarity increase, a hypsochromic shift of **12 a** is observed (i.e. negative solvatochromic).

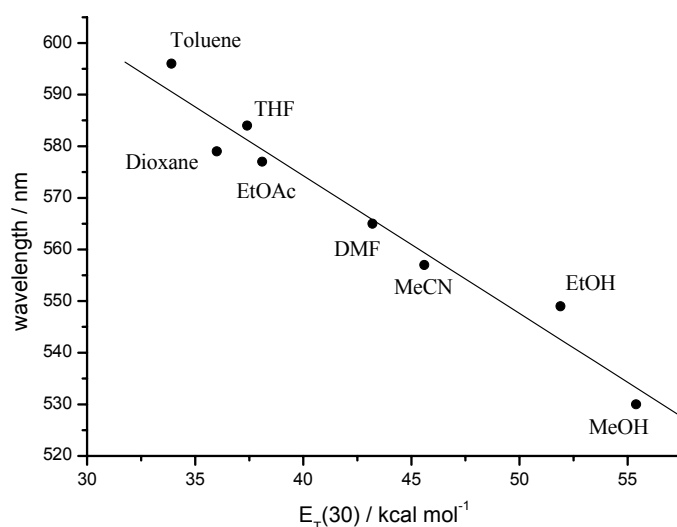


Figure 2.9. Linear plot on plotting λ_{\max} versus solvent parameter E_T

This observation indicates a highly polar ground state, i.e., one approaching the electronic distribution of the zwitterionic form. The observed negative photochromism of **12 a** implies that the ground state of the merocyanine form is relatively polar.^[43b] Hence, polar solvents will stabilize the ground state of the colored form more than the excited state. For further investigations of the system, theoretical calculations of π -dipole moments may be carried out. These dipole moments could then indicate qualitatively the polarities of the ground and first excited states.^[62]



Image 2.2. Visual representation of solvatochromism of **12 a**. Top image: after irradiation with visible light, Lower image: after irradiation with UV light; Solvents in both images: MeOH-DMF-EtOAc-Dioxane-THF-Toluene (from left to right).

Based on the relatively good stability of the merocyanine form, compared to the other spirobenzopyrans included in this series, the fluorescence spectra of **12 a** were measured in three different solvents. Therefore, solutions of the corresponding spirobenzopyran **12 a** were switched by irradiation with UV light into the merocyanine form until no further changes were observed and the solutions were directly measured. The merocyanines are excited (in MeOH: 526 nm, EtOH: 538 nm, MeCN: 553 nm) and show emission at 629 nm, 626 nm and 637 nm, respectively. Interestingly, these results reveal the large Stokes shift of the merocyanine form of **12 a** ($\Delta\lambda = 99$ nm, in MeOH).

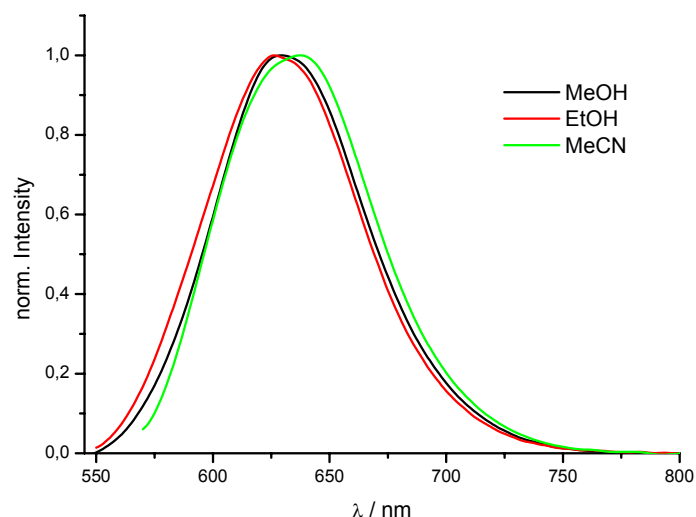


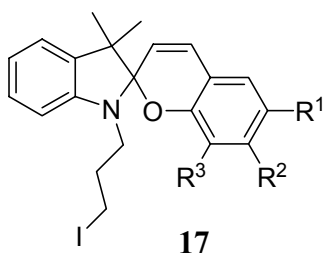
Figure 2.10. Fluorescence emission spectra of **12 a** (10 μ M) after irradiation with UV light ($\lambda = 312$ nm)



Image 2.3. Visual representation of red emission of **12 a** in MeOH during irradiation with UV light ($\lambda = 312$ nm)

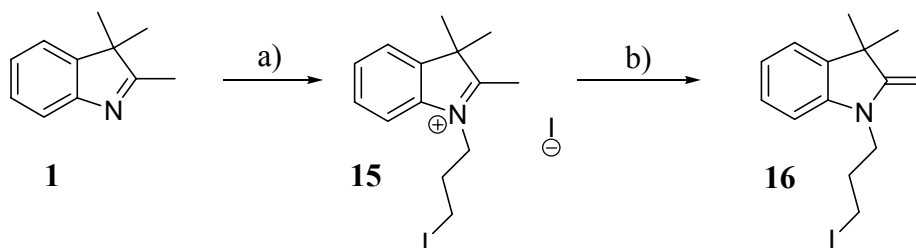
1.2.5. Synthesis of spirobenzopyrans and spiroxazine with linear C3-linker with iodo group

With use of the significantly facilitated and effective synthetic protocol that was used to survey the preparation of **12**, spirobenzopyrans with the general structure **17** were prepared (Scheme 2.10). The compounds are similar to **12**, but have an iodopropyl residue at the indole-N, instead of a propanol chain. This modification opens the possibility for practical transformations, e.g. into its azide or trimethylammonium derivatives.



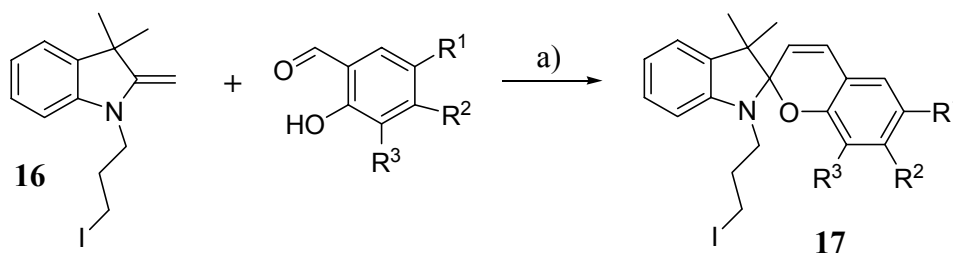
Scheme 2.10. General structure of spirobenzopyran **17** with substituent variations R^1 , R^2 , R^3

The N-alkylation of **1** with 1,3-diiodopropane was performed in acetonitrile under reflux for 48 hours and afforded **15** in 77 % yield. The indolium iodide **15** was subsequently deprotonated at the C2-methyl position with use of sodium hydroxide to afford the methyleneindoline **16** in 91 % yield (Scheme 2.11). Although, the product was successfully characterized by NMR and mass spectrometry, it needs to be mentioned that methyleneindolenine **16** is fairly unstable. Thus, **16** always needed to be freshly prepared for further use, otherwise formation of unwanted by-products occurred and led to very difficult separations and lowered yields.



Scheme 2.11. Synthesis of Iodopropylmethyleneindolenine **16**. Reagents and conditions: a) $I(CH_2)_3I$ (3.5 eq.), MeCN, reflux, 48 h, 77 %; b) NaOH (30 eq.), 80 °C to r.t., 1 h, 91 %.

Finally, a general procedure was used for the preparation of **17**. There, solutions of the freshly synthesized **16** and the corresponding salicylaldehydes were subjected to ultrasound radiation in ethanol (Scheme 2.12).



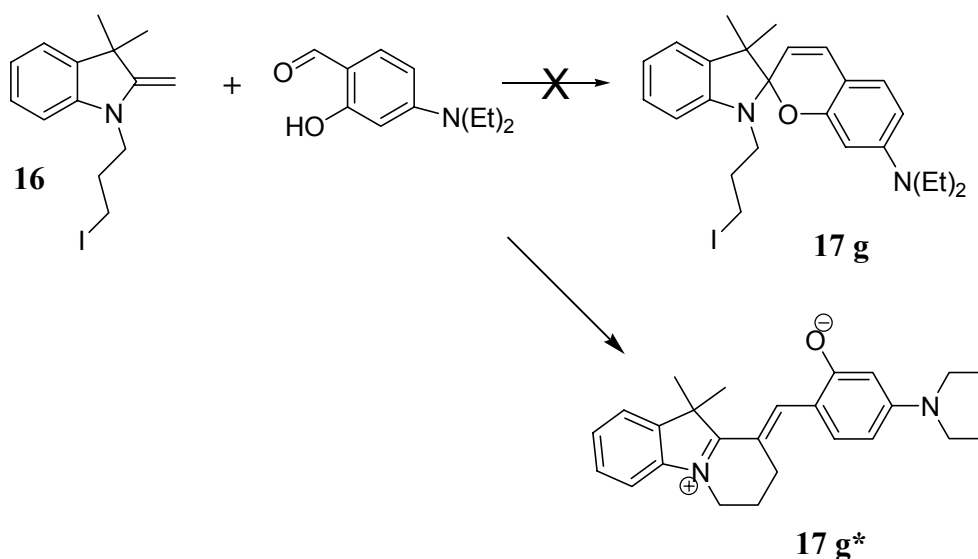
Scheme 2.12. General synthesis of **17**. Reagents and conditions: a) EtOH,))) , 50 - 65 min, 58-94 %

TLC was employed to check the reaction progress and revealed that the reaction succeeded after 50 to 65 minutes, followed by standard work-up procedure and purification by flash chromatography. The synthesized spirobenzopyrans **17 a** - **17 f** are listed below (Table 2.3).

Compound	R ¹	R ²	R ³	Yield (%)
17 a	NO ₂	H	H	94
17 b	Cl	H	H	74
17 c	Br	H	Br	83
17 d	Br	H	OCH ₃	80
17 e	NO ₂	H	OCH ₃	68
17 f	C≡CH	H	H	58

Table 2.3. Substituents and yields of spirobenzopyrans **17 a** - **17 f**

Interestingly, sonication of **16** with 5-(diethylamino)-2-hydroxybenzaldehyde did not yield the desired product **17 g**, but **17 g*** in 87 % yield (Scheme 2.13). It was formed already during the synthesis and detectable as a blazing pink spot on TLC plate. After chromatography, we were able to isolate and characterize **17 g*** by MS and 2D NMR experiments. In methanol, **17 g*** has λ_{max} at 542 nm and an emission maximum λ_{em} 585 nm.



Scheme 2.13. Formation of **17 g***. Reagents and conditions: EtOH, γ), 1 h, 87 %

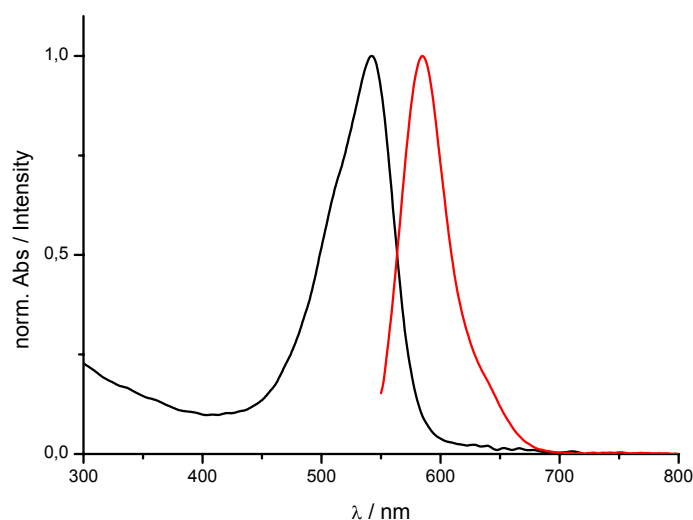
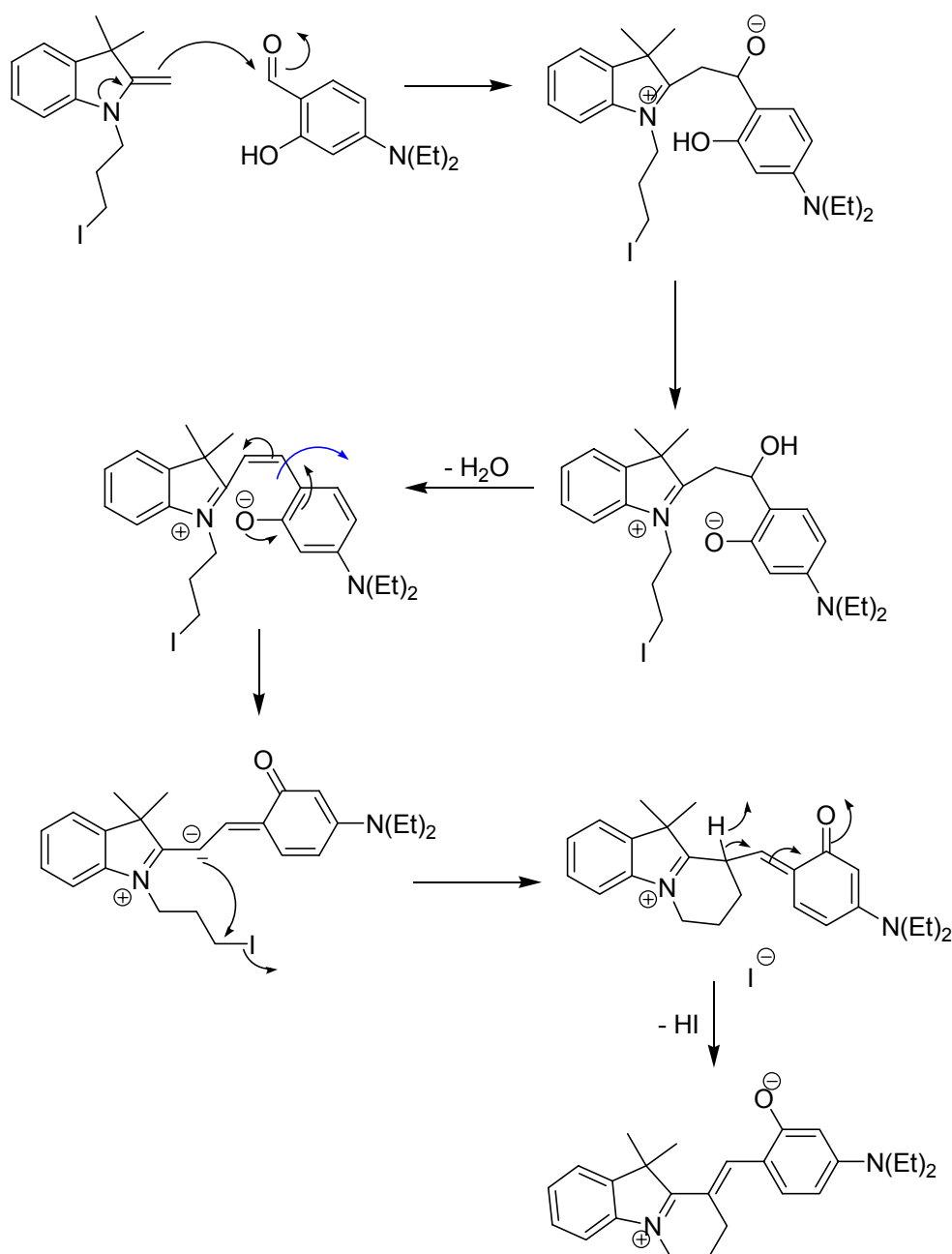


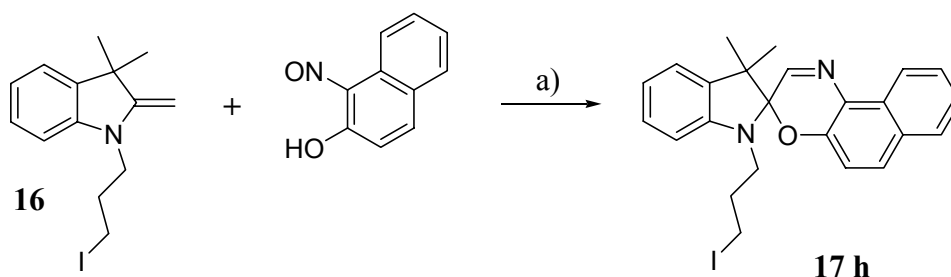
Figure 2.11. Normalized absorption and fluorescence emission spectra of **17 g*** in MeOH

Based on these results a hypothetical, but reasonable mechanism for the formation of **17 g*** is shown (Scheme 2.14). Nucleophilic addition of the heterocyclic enamine to the formyl group of the salicylaldehyde and a H-shift gives an adduct. Dehydration forms an isomer of the merocyanine. After charge redistribution this leads to a nucleophilic attack of the potentially negative carbon to cleave off I⁻ and formation of the six-ring. Finally, the concerted rearomatization and elimination of HI lead to the zwitterionic **17 g***.



Scheme 2.14. Supposed mechanism for the formation of **17 g***

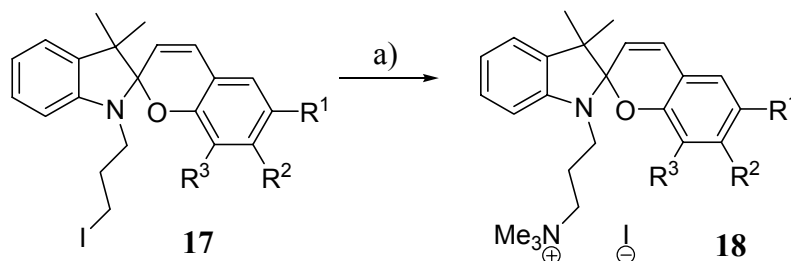
The application of ultrasonic irradiation was also used to prepare the iodopropyl-substituted spirooxazine derivative **17 h**. The reaction was carried out in ethanol, where **16** and 1-nitroso-2-naphthol (1.1 eq.) were irradiated for an overall of 2 hours with ultrasound (Scheme 2.15). Unlike the formation of the spirobenzopyrans **17 a - 17 f**, unreacted starting material was still found in the crude product. Since further product formation was not observed after 2 hours, we may assume that experiments with change of the ultrasound frequency may lead to a higher reaction rate and better yields. However, after flash chromatography the desired product **17 h** was obtained as a yellow powder in 25 % yield.



Scheme 2.15. Synthesis of spiroxazine **17 h**. Reagents and conditions: a) EtOH, γ), 2 h, 25 %

1.2.6. Functional transformation using trimethylamine

For the study of non-covalent spirobenzopyran DNA interactions use of trimethylammonium equipped spirobenzopyrans has been suggested.^[63] For future studies we therefore substituted the iodide residues of **17 a** - **17 e** successfully with the cationic trimethylammonium residue (Table 2.4). There, the iodopropyl-spirobenzopyrans **17** were stirred in an ethanol solution with excess of trimethylamine (66 eq.) for 60 - 64 hours (Scheme 2.16). The products crystallized nicely and were isolated in high yields.



Scheme 2.16. General synthesis of **18**. Reagents and conditions: a) NMe₃ (66 eq.), EtOH, r.t., 60-64 h, 79-91 %

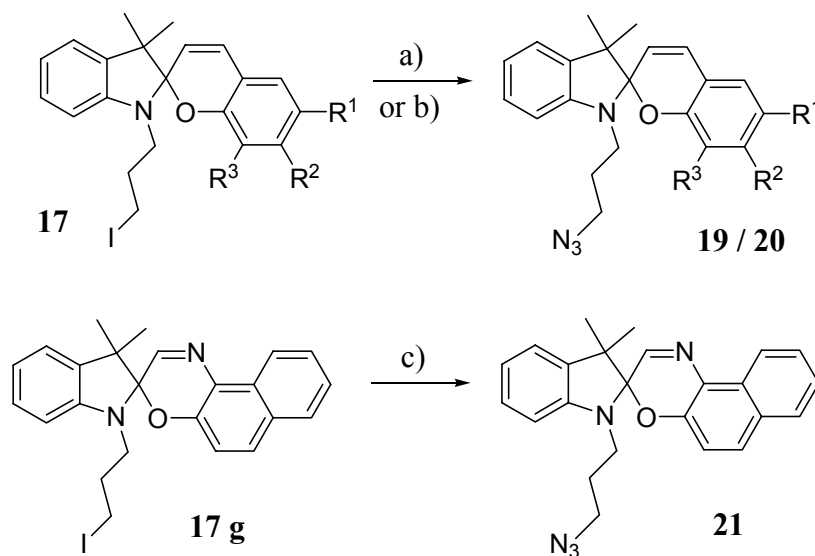
Compound	R ¹	R ²	R ³	Yield (%)
18 a	NO ₂	H	H	79
18 b	Cl	H	H	91
18 c	Br	H	Br	88
18 d	Br	H	OCH ₃	85
18 e	NO ₂	H	OCH ₃	89

Table 2.4. Substituents and yields of spirobenzopyrans **18 a** - **18 e**

1.2.7. Synthesis of spirobenzopyran and spiroxazine azides

To apply spirobenzopyran and spiroxazine systems as photochromic labels, functional groups need to be attached to them. One of the most popular functional groups used for a bio-orthogonal labeling strategy are terminal alkynes and azides.^[64] Colloquial use of the term “click”-reactions mainly refers to the established procedures for 1,4-regioselective preparation of triazoles, by reacting alkynes and azides under copper-(I) catalysis.^[65] Beside the aspect of bio-orthogonality, the “click”-reactions also provide a broad range for the coupling of azides and functional π -systems, often bearing alkynyl functionalities and will be further discussed in Chapter 2.

Thus, the three azides of potentially interesting photo-responsive molecules were synthesized. Therefore, the corresponding iodides were subjected to an excess of sodium azide in dimethylformamide and stirred at room temperature. After nucleophilic substitution, the desired products were obtained in very good yields (Scheme 2.17).



Scheme 2.17. Synthesis of spiropyran / spirooxazine azides. Reagents and conditions: a) for **19** ($R^1 = \text{NO}_2$, $R^2, R^3 = \text{H}$), NaN_3 (4.1 eq.), DMF, r.t., 19 h, 83 %; b) for **20** ($R^1 = \text{C}\equiv\text{CH}$, $R^2, R^3 = \text{H}$), NaN_3 (5.7 eq.), DMF, r.t., 48 h, 96 %; c) for **21**, NaN_3 (4.0 eq.), DMF, r.t., 48 h, 84 %.

In addition to their synthetic access, the applicability of the spirobenzopyran azide **19** for the preparation of molecular dyads (Chapter 2) and labeling of DNA (Chapter 3) was also investigated.

1.3. Conclusion

Several new spirobenzopyrans and spiroxazines were synthesized and the reaction yields were improved with use of ultrasonic irradiation. Functional modifications at the photochromic molecules were introduced using an alkyl linker bearing hydroxyl or iodo groups at the N-indole sections or carboxylic acid incorporation, also at the indole moiety. The compounds were also equipped with trimethylammonium, azido and ethynyl groups that could be useful in combination with functional π -systems or biopolymers. The reversibility of the photoswitching was demonstrated for representative compounds. Influence of different substitution patterns at the phenolic moiety have been discussed, including electron-withdrawing and -donating substituents. The visible absorption spectra of the corresponding merocyanines formed by photo-induced ring opening were examined. The solvatochromic behaviour of a merocyanine with a strong electron-withdrawing group was examined in various solvents and showed negative solvatochromism.

1.4. Experimental Section

Reagents, solvents and reaction processing

Unless otherwise specified, reagents and starting materials were purchased from commercial suppliers and used without further purification. $\text{PdCl}_2(\text{PPh}_3)_2$ was prepared according to the literature.^[66] Dry solvents were prepared according to procedures reported in the literature.^[53a, 67] Unless otherwise emphasized, all reactions were carried out at room temperature in degassed solvents and under N_2 or Argon atmosphere. Reactions using ultrasound were carried out in deoxygenated solvents with use of a Sonorex Super RK510 ultrasonic system with superaudio frequency of 35 kHz. Synthetic steps involving indole or spirobenzopyran units were performed in the dark or under fluorescent lamps (Conrad Elektronik 590208 - 62).

Chromatography

Flash chromatography and dry column flash chromatography were performed on silica gel (Merck Silica Gel Si 60 40-63 μm and Acros silica gel, 35-70 μm , 60 Å) according to the reported methods.^[53a, 54a, 55, 68] Preparative TLC was carried out on glass plates coated with silica gel (Merck Silica Gel 60, 20 x 20 cm, F₂₅₄, thickness 2mm). TLC was carried out with use of alumina plates coated with silica gel (Merck Silica Gel 60 F₂₅₄, thickness 0.25 mm) and

compounds were visualized by UV light ($\lambda = 254, 366$ nm), MeOH/H₂SO₄ stain (5 vol-% H₂SO₄) and standard staining solutions (p-Anisaldehyde stain, Vanillin Stain, Phosphomolybdic acid stain).^[53a]

NMR

NMR spectra were measured at the University of Regensburg, Zentrale Analytik, on Bruker Avance 300 (¹H: 300.1 MHz, ¹³C: 75.5 MHz, T = 300 K), Bruker Avance 400 (¹H: 400.1 MHz, ¹³C: 100.6 MHz, T = 300 K) and Bruker Avance 600 (¹H: 600.1 MHz, ¹³C: 150.1 MHz, T = 300 K) instruments. The solvent for each spectrum is reported. Chemical shifts are reported in δ /ppm relative to external standards and coupling constants J are given in Hz. Signal characterization: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = double doublet, dt = double triplet, ddd = double double doublet. The solvent used is reported for each spectrum. The relative number of protons is determined by integration. Error of reported values: 0.01 ppm (¹H NMR), 0.1 ppm (¹³C NMR), coupling constant 0.1 Hz. The assignment of ¹H NMR spectra was aided by COSY experiments. The assignment of ¹³C NMR spectra was aided by DEPT 90, DEPT 135, HSQC, HMBC and NOESY experiments.

MS

Mass spectra measurements were performed at the University of Regensburg, Zentrale Analytik Massenspektrometrie, with Finnigan MAT SSQ 710 A (EI-MS and CI-MS), ThermoQuest Finnigan TSQ 7000 (ESI-MS), Finnigan MAT 95 (EI-MS, FAB-MS and HRMS).

IR

IR spectra were recorded with a Bio-Rad FT-IR Excalibur FTS 3000 MX spectrometer.

UV/Vis and fluorescence spectroscopy

Unless otherwise specified, spectroscopic measurements were performed at 20 °C and quartz glass cuvettes (Starna, 10 mm) were used. UV/Vis spectra were recorded with a Cary BIO 50 and Cary BIO 100 UV/Vis/NIR spectrometer (Varian) with temperature-controlled 6x6 cuvette holder. Fluorescence measurements were performed with a Fluoromax-3 Fluorimeter from Jobin-Yvon with slit width 2-5 nm, with Peltier-element (LFI-3751) for temperature control and are corrected for the Raman emission from the solution.

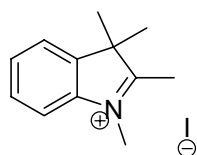
X-ray crystallography

The X-ray crystal structure measurement was performed at the University of Regensburg, Zentrale Analytik NWF IV, with use of a STOE-IPDS diffractometer. Further details are given in the appendix.

Light sources

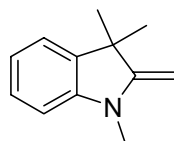
For irradiation experiments a UV hand-held lamp (Herolab, 6 W, $\lambda = 312$ nm), a UV hand-held lamp (Faust, 2 x 4 W, $\lambda = 366$ nm) and a Luxeon III Star high-power LED ($\lambda = 590$ nm / amber) were used.

1,2,3,3-tetramethyl-3H-indolium iodide



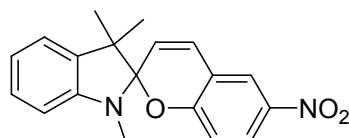
Freshly distilled 2,3,3-Trimethylindolenine (2.38 mL, 14.88 mmol) was dissolved in dry MeCN (9.0 mL) and Iodomethane (1.0 mL, 16.16 mmol) was added. The mixture was refluxed under nitrogen atmosphere at 85 °C for 24 hours. Following, the mixture was stirred at room temperature for another two hours and cooled to 5 °C. The pink reaction mixture was filtered and the remaining precipitate was washed repeatedly with hexane, chloroform and ether until the crystals were ivory. Following, the crystals were triturated with Et₂O and dried in a desiccator to afford 1,2,3,3-tetramethyl-3H-indolium iodide as a white fuzzy powder (3.312 g, 74 %).

¹H NMR (300 MHz, DMSO-d₆) δ = 7.96-7.89 (m, 1 H, H-Ar), 7.87-7.80 (m, 1 H, H-Ar), 7.67-7.57 (m, 2 H, H-Ar), 3.99 (s, 3 H, NCH₃), 2.79 (s, 3 H, 2-Me), 1.54 (s, 6 H, 3-Me) - ¹³C NMR (75 MHz, DMSO-d₆) δ = 195.9 (C_{quat.}), 142.0 (C_{quat.}), 141.5 (C_{quat.}), 129.2 (+, CH), 128.7 (+, CH), 123.2 (+, CH), 115.1 (+, CH), 53.8 (C_{quat.}), 34.8 (+, CH₃), 21.6 (+, CH₃, C(CH₃)₂), 14.3 (+, CH₃) - MS (ESI): m/z (%): 174.0 (100) [M⁺]

1,3,3-trimethyl-2-methyleneindoline

1,2,3,3-tetramethyl-3H-indolium iodide (3.500 g, 11.63 mmol) and an aqueous KOH solution (38.8 mmol, 38.8 mL) were stirred at room temperature for 3 hours. Following, the solution was extracted with Et₂O (120 mL), the organic layer was dried over anhydrous MgSO₄ and the solvent was removed under reduced pressure. The remaining red oil was purified by distillation to afford 1,3,3-trimethyl-2-methyleneindoline as a colorless oil (1.564 g, 77 %).

¹H NMR (300 MHz, CDCl₃) δ = 7.17-7.07 (m, 2 H, H-Ar), 6.76 (dd, 1 H, J = 4.2, 10.6 Hz, H-Ar), 6.55 (d, 1 H, J = 7.8 Hz, H-Ar), 3.85 (s, 2 H, 2-CH₂), 3.05 (s, 3 H, NCH₃), 1.36 (s, 6 H, 3-CH₃) - MS (EI, 70 eV): m/z (%): 160.2 (100) [M⁺-CH₃·], 175.2 (63) [M⁺·]

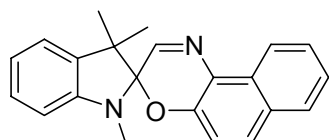
1',3'-dihydro-1',3',3'-trimethyl-6-nitro-spiro[2H-1-benzopyran-2,2'-[2H]indole]**Route A:**

A Schlenk flask was charged with a solution of 2-hydroxy-5-nitrobenzaldehyde (1.510 g, 9.035 mmol) in dry EtOH (40 mL), degassed and heated to reflux. Following, 1,3,3-trimethyl-2-methyleneindoline (1.564 g, 9.04 mmol) in dry EtOH (5.0 mL) was added over 5 minutes. The reaction mixture was refluxed for 4 hours, then under vigorous stirring allowed to cool to room temperature and stirred for additional 14 hours. The solvent was removed under reduced pressure and the remaining purple-red solid was purified by column chromatography on silica gel (acetone). Finally, the purified orange-brown solid was triturated with Et₂O to afford 1',3'-dihydro-1',3',3'-trimethyl-6-nitro-spiro[2H-1-benzopyran-2,2'-[2H]indole] as pale red crystals (2.818 g, 97 %).

Route B:

A flask was capped with rubber septa and flushed with nitrogen. The flask was charged with 1,3,3-trimethyl-2-methyleneindoline (1.125 g, 6.43 mmol) and dry EtOH (52 mL). Furthermore, 2-hydroxy-5-nitrobenzaldehyde (1.077 g, 6.44 mmol) was added and the solution was degassed. The mixture was sonicated at 35 kHz for 80 minutes, the product was allowed to crystallize in the freezer, filtered off and washed with cold EtOH. The precipitate was dried in vacuo and the remaining residue was recrystallized in EtOAc to yield 1',3'-dihydro-1',3',3'-trimethyl-6-nitro-spiro[2H-1-benzopyran-2,2'-[2H]indole] as pale red crystals (1.946 g, 94 %).

^1H NMR (300 MHz, CDCl_3) δ = 8.00 (m, 2 H, H-Ar), 7.20 (m, 1 H, H-Ar), 7.09 (d, 1 H, J = 7.2 Hz, H-Ar), 6.90 (m, 2 H, H-Ar), 6.75 (d, 1 H, H-Ar), 6.56 (d, 1 H, H-Ar), 5.86 (d, 1 H, H-Ar), 2.75 (s, 3 H, NCH_3), 1.30 (s, 3 H, 3- CH_3), 1.20 (s, 3 H, 3- CH_3) - MS (EI, 70 eV): m/z (%): 322.0 (100) [M^+], 307.0 (36) [$\text{M}^+ - \text{CH}_3$]

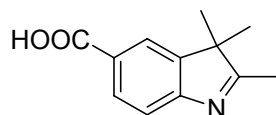
1,3-dihydro-1,3,3-trimethyl-spiro[2H-indole-2,3'-[3H]naphth[2,1-b][1,4]oxazine]

A Schlenk flask was charged with 1-Nitroso-2-naphthol (808 mg, 4.67 mmol) and dry MeOH (50 mL). The solution was deaerated and freshly prepared 1,3,3-trimethyl-2-methyleneindoline (825 μL , 4.66 mmol) was added under a nitrogen atmosphere. The mixture was sonicated for 2 hours at 35 kHz, then the solvent was removed under reduced pressure and the residue was dried in vacuo. Purification by gradient flash chromatography on silica gel (Hexane/THF 30:1 to 20:1) afforded 1,3-dihydro-1,3,3-trimethyl-spiro[2H-indole-2,3'-[3H]naphth[2,1-b][1,4]oxazine as a lustrous orange solid (995 mg, 65 %). R_f = 0.25 (Hexane/THF 25:1)

^1H NMR (300 MHz, CDCl_3): δ = 8.57 (d, 1 H, J = 8.5 Hz), 7.75 (d, 2 H, J = 6.2 Hz), 7.67 (d, 1 H, J = 8.9 Hz), 7.59 (ddd, 1 H, J = 1.2, 6.9, 8.3 Hz), 7.41 (ddd, 1 H, J = 1.2, 6.9, 8.1 Hz),

7.22 (dd, 1 H, $J = 1.2, 7.7$ Hz), 7.10 (dd, 1 H, $J = 0.9, 7.2$ Hz), 7.02 (d, 1 H, $J = 8.9$ Hz), 6.91 (dt, 1 H, $J = 0.7, 7.4$ Hz), 6.59 (d, 1 H, $J = 7.8$ Hz), 2.78 (s, 3 H), 1.37 (s, 3 H), 1.36 (s, 3 H) - ^{13}C NMR (75 MHz, CDCl_3): $\delta = 150.8$ (+, CH), 147.6 ($\text{C}_{\text{quat.}}$), 144.1 ($\text{C}_{\text{quat.}}$), 135.9 ($\text{C}_{\text{quat.}}$), 130.8 ($\text{C}_{\text{quat.}}$), 130.3 (+, CH), 129.3 ($\text{C}_{\text{quat.}}$), 128.0 (+, CH), 127.8 (+, CH), 127.1 (+, CH), 124.2 (+, CH), 122.9 ($\text{C}_{\text{quat.}}$), 121.5 (+, CH), 119.9 (+, CH), 116.8 (+, CH), 110.9 (+, CH), 107.2 (+, CH), 98.6 ($\text{C}_{\text{quat.}}$), 51.8 ($\text{C}_{\text{quat.}}$), 29.7 (+, CH_3), 25.5 (+, CH_3), 20.8 (+, CH_3) - MS (CI, 70 eV): m/z (%): 328.2 (100) [M^+], 313.1 (76) [$\text{M}^+ - \text{CH}_3$] - HRMS (EI-MS) calcd. for $\text{C}_{23}\text{H}_{20}\text{N}_2\text{O}$ [M^+]: 328.1576, found: 328.1573

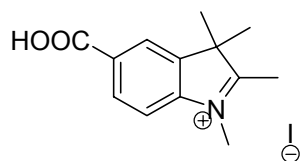
2,3,3-trimethyl-3H-indole-5-carboxylic acid



To a solution of 4-hydrazinylbenzoic acid (11.702 g, 76.91 mmol) in EtOH (23 mL), 3-methyl-2-butanone (9.1 mL, 85.05 mmol) and concentrated H_2SO_4 (2.2 mL) were added and the reaction mixture was heated under reflux for 16 hours. The solution was allowed to cool to ambient temperature and filtered. The filtrate was concentrated in vacuo to its half volume, washed with saturated aqueous K_2CO_3 solution (200 mL) and extracted with CH_2Cl_2 (2 x). The aqueous layer was extracted with Et_2O and the organic layers were pooled. Removal of the solvents gave an amber-orange oil and concentrated HCl was added until pH 5 to precipitate the product. 2,3,3-trimethyl-3H-indole-5-carboxylic acid was obtained as orange-yellow solid (9.916 g, 63 %).

MS (EI, 70 eV): 203.1 (100) [M^+]

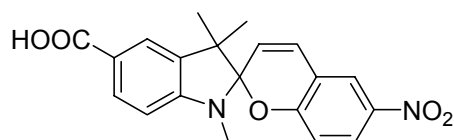
5-carboxy-1,2,3,3-tetramethyl-3H-Indolium iodide



A solution of 2,3,3-trimethyl-3H-indole-5-carboxylic acid (3.305 g, 16.27 mmol) in CHCl_3 (22 mL) was treated with iodomethane (2.1 mL, 33.73 mmol) and held at reflux for 18 hours. The mixture was slowly cooled to 0 °C, diluted by addition of cold hexane and filtered. The precipitate was washed with cold hexane (20 mL), dried in vacuo and 5-carboxy-1,2,3,3-tetramethyl-3H-Indolium iodide was obtained as a pale fawn solid (4.579 g, 81 %)

^1H NMR (300 MHz, DMSO-d_6): δ = 8.38 (s, 1 H, arom., H-4), 8.19 (d, 1 H, J = 8.3 Hz, arom. H-6), 8.02 (d, 1 H, J = 8.4 Hz, arom. H-7), 3.99 (s, 3 H, NCH_3), 2.81 (s, 3 H, CH_3), 1.56 (s, 6 H, 2 x CH_3) - MS (ESI): m/z (%): 221.1 (100) $[\text{MH}^+]$, 262.1 (37) $[\text{MH}^+ + \text{MeCN}]$

1',3'-dihydro-1',3',3'-trimethyl-6-nitro-spiro[2H-1-benzopyran-2,2'-[2H]indole]-5'-carboxylic acid



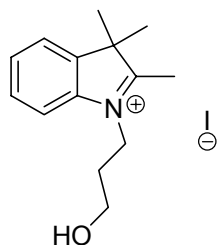
Route A:

A Schlenk flask was charged with 5-carboxy-1,2,3,3-tetramethyl-3H-Indolium iodide (592 mg, 1.72 mmol), dry EtOH (23 mL) and dry piperidine (170 μL , 1.72 mmol) under an argon atmosphere. Following, 2-hydroxy-5-nitrobenzaldehyde (285 mg, 1.71 mmol) was added and the solution was degassed. The reaction mixture was refluxed for 18 hours, then very slowly cooled to room temperature and in addition placed in a freezer over night to give an olive-green precipitate. The solvent was filtered off and the precipitate was washed with cold EtOH and MeOH. Finally, the solid was recrystallized in EtOH and dried in vacuo to yield 1',3'-dihydro-1',3',3'-trimethyl-6-nitro-spiro[2H-1-benzopyran-2,2'-[2H]indole]-5'-carboxylic acid as smooth orange-green crystals (588 mg, 94 %).

Route B:

A flask was capped with rubber septa and flushed with argon. The flask was charged with 5-carboxy-1,2,3,3-tetramethyl-3H-Indolium iodide (340 mg, 0.985 mmol) and dry EtOH (11 mL). Furthermore, piperidine (120 μ L, 1.212 mmol) and 2-hydroxy-5-nitrobenzaldehyde (164 mg, 0.981 mmol) were added and the solution was degassed via freeze-pump-thaw (3 cycles). The mixture was sonicated at 35 kHz for 60 minutes, then the solvents were removed under reduced pressure, and the remaining residue was triturated over night with Et₂O to yield 1',3'-dihydro-1',3',3'-trimethyl-6-nitro-spiro[2H-1-benzopyran-2,2'-[2H]indole]-5'-carboxylic acid as subtle orange-green crystals (354 mg, 98 %).

¹H NMR (300 MHz, DMSO-d₆): δ = 8.15 (d, 1 H, J = 2.8 Hz, H-5'), 7.99 (dd, 1 H, J = 2.8, 9.0 Hz, H-7'), 7.81 (dd, 1 H, J = 1.6, 8.2 Hz, H-8'), 7.65 (d, 1 H, J = 1.6 Hz, H-4), 7.17 (d, 1 H, J = 10.4 Hz, H-4'), 6.85 (d, 1 H, J = 9.0 Hz, H-6), 6.64 (d, 1 H, J = 8.2 Hz, H-7), 5.96 (d, 1 H, J = 10.4 Hz, H-3'), 2.75 (s, 3 H, NCH₃), 1.23 (s, 3 H, CH₃), 1.12 (s, 3 H, CH₃) - ¹³C NMR (75 MHz, DMSO-d₆): δ = 158.8 (COOH), 151.1 (C_{quat.}), 135.7 (C_{quat.}), 130.6 (+, CH), 128.2 (+, CH), 125.4 (+, CH), 122.6 (+, CH), 122.4 (+, CH), 121.3 (C_{quat.}), 120.5 (+, CH), 118.5 (C_{quat.}), 115.1 (+, CH), 105.8 (+, CH), 105.7 (C_{quat.}), 51.3 (C_{quat.}), 47.2 (C_{quat.}), 38.6 (C_{quat.}), 27.7 (+, CH₃), 24.9 (+, CH₃), 18.9 (+, CH₃) - IR (neat): ν [cm⁻¹] = 2970, 2359, 1738, 1366, 1350, 1228, 1217 - MS (ESI): m/z (%): 367.0 (100) [MH⁺], 408.0 (21) [MH⁺+MeCN]

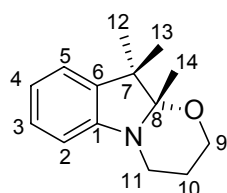
1-(3-Hydroxypropyl)-2,3,3-trimethyl-3H-indolium iodide

Under nitrogen atmosphere freshly distilled 2,3,3-Trimethylindolenine (1.5 mL, 9.34 mmol) was dissolved in chloroform (17 mL). The solution was degassed via freeze-pump-thaw (3 cycles), 3-Iodo-1-propanol (1.00 g, 10.44 mmol) was added and refluxed under nitrogen for 24 hours. The reaction mixture was slowly cooled to room temperature and the solvent was removed under reduced pressure. The remaining purple oil was washed with petrolether and

trituated with Et₂O to afford 1-(3-Hydroxypropyl)-2,3,3-trimethyl-3H-indolium iodide as a purple solid (3.224 g, 99 %).

¹H NMR (DMSO-d₆, 300 MHz): δ = 8.01-7.91 (m, 1 H), 7.90-7.81 (m, 1 H), 7.67-7.57 (m, 2 H), 4.56 (t, 2 H, J = 6.9 Hz), 3.55 (t, 2 H, J = 5.5 Hz), 2.87 (s, 3 H), 2.11-1.97 (m, 2 H), 1.54 (s, 6 H) - ¹³C NMR (DMSO-d₆, 75 MHz): δ = 196.4 (C_{quat.}), 141.7 (C_{quat.}), 141.0 (C_{quat.}), 129.2 (+, CH), 128.8 (+, CH), 123.4 (+, CH), 115.4 (+, CH), 57.8 (-, CH₂), 54.1 (C_{quat.}), 45.7 (-, CH₂), 29.6 (-, CH₂), 21.8 (+, CH₃), 14.3 (+, CH₃), 5.4 (+, CH₃) - MS (ESI, DCM/MeOH + 10 mmol/L NH₄Ac): m/z (%) = 218.0 (100) [M⁺]

(S)-3,4,10,10a-Tetrahydro-10,10,10a-trimethyl-2H-[1,3]oxazino[3,2-a]indole

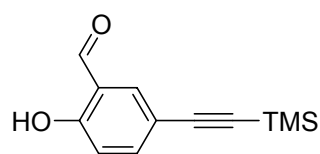


1-(3-Hydroxypropyl)-2,3,3-trimethyl-3H-indolium iodide (3.093 g, 8.97 mmol) was suspended in degassed water (53 mL) and finely ground potassium hydroxide (1.226 g, 21.85 mmol) was added. The reaction mixture was stirred at room temperature under nitrogen for 2 hours. Dichloromethane (50 mL) was added and the mixture was stirred for additional 30 minutes. The aqueous layer was separated and extracted with dichloromethane (2 x 45 mL). The combined organic layers were washed with brine, water and dried over anhydrous Na₂SO₄. The solvent was removed under reduced pressure and the residue was dried under vacuum. The crude product was purified by gradient flash chromatography on silica gel (Hexane/EtOAc 4:1 to 1:1) to afford (S)-3,4,10,10a-Tetrahydro-10,10,10a-trimethyl-2H-[1,3]oxazino[3,2-a]indole as colorless crystals (0.835 g, 43 %). R_f = 0.47 (Hexane/EtOAc 4:1). Crystals suitable for X-ray analysis were grown by slow evaporation of EtOAc at r.t. from a concentrated solution of the purified product.

¹H NMR (600 MHz, CDCl₃): δ = 7.13 (t, 1 H, J = 7.7 Hz, CH-C3), 7.08 (d, 1 H, J = 7.3 Hz, CH-C5), 6.81 (t, 1 H, J = 7.2 Hz, CH-C4), 6.59 (d, 1 H, J = 7.8 Hz, CH-C2), 4.08 (dt, 1 H, J = 2.6, 12.4 Hz, CH₂-C9), 3.72 (dd, 1 H, J = 5.2, 11.8 Hz, CH₂-C9), 3.66 (dd, 1 H, J = 4.6, 14.6

Hz, CH₂-C11), 3.54 (m, 1 H, CH₂-C11), 2.02-1.92 (m, 1 H, CH₂-C10), 1.56 (s, 3 H, CH₃-C14), 1.30 (s, 3 H, CH₃-C12), 1.20 (d, 1 H, J = 13.4 Hz, CH₂-C10), 1.07 (s, 3 H, CH₃-C13) - ¹³C NMR (150 MHz, CDCl₃): δ = 148.1 (C1), 139.2 (C6), 127.2 (C3), 122.0 (C5), 119.2 (C4), 108.6 (C2), 98.3 (C8), 61.0 (C9), 48.0 (C7), 39.1 (C11), 26.7 (C13), 21.7 (C10), 18.6 (C12), 13.0 (C14) - MS (CI, 70 eV): m/z (%): 218.2 (100) [MH⁺] - HRMS (EI-MS) calcd. for C₁₄H₁₉NO [M⁺]: 217.1467, found: 217.1472

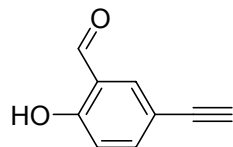
2-Hydroxy-5-(2-(trimethylsilyl)ethynyl)benzaldehyde



A Schlenk flask was charged with 5-Bromosalicylaldehyde (1.529 g, 7.61 mmol) and dry NEt₃ (28.0 mL) under an atmosphere of N₂ and the solution was stirred for 5 minutes at room temperature. Following, 170 mg PdCl₂(PPh₃)₂ (170 mg, 0.24 mmol) and CuI (50 mg, 0.26 mmol) were added and the solution was degassed. Under a slow flow of argon Ethynyltrimethylsilane (1.66 mL, 11.98 mmol) was added quickly and the reaction mixture was heated at 80 °C for 3 hours. The reaction mixture was slowly cooled to room temperature, dry THF (80 mL) was added and stirred at room temperature for 1 hour. The mixture was concentrated in vacuo, diluted with CH₂Cl₂, washed with water. The aqueous phase was extracted with CH₂Cl₂, the organic layers were combined and dried over anhydrous Na₂SO₄. The solvent was evaporated and the remaining solid was purified by gradient flash chromatography on silica gel (Hexane/THF 40:1 to 10:1) to yield 2-Hydroxy-5-(2-(trimethylsilyl)ethynyl)benzaldehyde as colorless crystals (1.452 g, 88 %). R_f = 0.25 (Hexane/THF 40:1)

¹H NMR (300 MHz, CDCl₃): δ = 11.13 (s, 1 H), 9.88 (s, 1 H), 7.73 (d, 1 H, J = 2.0 Hz), 7.63 (dd, 1 H, J = 2.1, 8.7 Hz), 6.96 (d, 1 H, J = 8.7 Hz), 0.28 (s, 9 H) - ¹³C NMR (CDCl₃, 75 MHz): 196.1 (+, CH), 161.6 (C_{quat.}), 140.2 (+, CH), 137.5 (+, CH), 120.4 (C_{quat.}), 118.0 (+, CH), 115.2 (C_{quat.}), 103.2 (C_{quat.}), 93.9 (C_{quat.}), 0.0 (+, CH₃) - IR (neat): ν [cm⁻¹] = 2970, 2360, 1738, 1466, 1373 - MS (EI, 70 eV): m/z (%): 218.0 (32) [M⁺], 203.0 (100) [M⁺-CH₃] - HRMS (PI-MS) calcd. for C₁₂H₁₄O₂Si [M⁺]: 218.0763, found: 218.0766

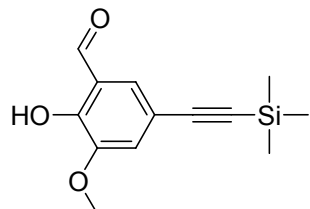
5-Ethynyl-2-hydroxybenzaldehyde



To a solution of 2-Hydroxy-5-(2-(trimethylsilyl)ethynyl)benzaldehyde (1.396 g, 6.40 mmol) in dry THF (40 mL), a freshly prepared solution of Bu₄NF in dry THF (12 mL, 12.00 mmol) was added. The reaction mixture was stirred at room temperature for 30 minutes, and water (35 mL) was added. The mixture was extracted with Et₂O (2 x 40 mL), the yellow-green organic layers were combined and dried over anhydrous Na₂SO₄, the solution was filtered and concentrated in vacuo. Purification was performed by flash chromatography on silica gel (Hexane/THF 20:1) and 5-Ethynyl-2-hydroxybenzaldehyde was isolated as a ivory-colored solid (860 mg, 92 %). R_f = 0.35 (Hexane/THF 20:1)

¹H NMR (300 MHz, CDCl₃): δ = 11.15 (s, 1 H), 9.88 (s, 1 H), 7.74 (d, 1 H, J = 2.1 Hz), 7.64 (dd, 1 H, J = 2.1, 8.7 Hz), 6.98 (d, 1 H, J = 8.7 Hz), 3.06 (s, 1 H) - ¹³C NMR (75 MHz, CDCl₃): δ = 195.0 (+, CH), 160.7 (C_{quat.}), 139.2 (+, CH), 136.5 (+, CH), 119.4 (+, CH), 117.1 (C_{quat.}), 112.9 (C_{quat.}), 80.8 (+, CH), 28.7 (C_{quat.}) - MS (EI, 70 eV): m/z (%): 146.1 (100) [M⁺], 145.1 (54) [M⁺-H], 117.1 (10) [M-CHO⁺]

2-Hydroxy-3-methoxy-5-(2-(trimethylsilyl)ethynyl)benzaldehyde

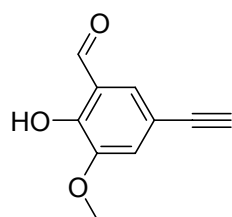


A Schlenk flask was charged under argon atmosphere with 5-Bromo-2-hydroxy-3-methoxybenzaldehyde (934 mg, 4.04 mmol), PdCl₂(PPh₃)₂ (97 mg, 0.14 mmol) and CuI (30 mg, 0.16 mmol), and dry NEt₃ (18 mL) was added. The mixture was degassed via freeze-

pump-thaw (3 cycles) and Ethynyltrimethylsilane (0.88 mL, 6.35 mmol) was added under argon atmosphere. The reaction mixture was heated at 80 °C for 3 hours, slowly cooled to room temperature, CH₂Cl₂ (20 mL) and water (20 mL) were added and the mixture was stirred for 30 minutes. The mixture was extracted with CH₂Cl₂, washed with brine and water and the aqueous layer was extracted with CH₂Cl₂. The organic layers were combined and dried over anhydrous MgSO₄, filtered and the solvent was evaporated under reduced pressure. The residue was dried in vacuo over night and the remaining solid was purified by flash chromatography on silica gel (CH₂Cl₂/MeOH 20:1) to give 2-Hydroxy-3-methoxy-5-(2-(trimethylsilyl)ethynyl)benzaldehyde as fawn crystals (730 mg, 73 %). *R*_f = 0.25 (CH₂Cl₂)

¹H NMR (300 MHz, CDCl₃): δ = 11.23 (s, 1 H), 9.87 (s, 1 H), 7.35 (d, 1 H, *J* = 1.8 Hz), 7.16 (d, 1 H, *J* = 1.5 Hz), 3.92 (s, 3 H), 0.58 (s, 9 H) - ¹³C (75 MHz, CDCl₃): δ = 196.2 (+, CH), 152.1 (C_{quat.}), 148.2 (C_{quat.}), 128.6 (+, CH), 120.4 (+, CH), 120.4 (C_{quat.}), 114.7 (C_{quat.}), 93.7 (C_{quat.}), 56.4 (+, CH₃), 29.8 (C_{quat.}), 0.0 (+, CH₃) - MS (EI, 70 eV): *m/z* (%): 249.0 (87) [MH⁺], 266.1 (100) [M+NH₄⁺] - HRMS (PI-MS) calcd. for C₁₃H₁₆O₃Si [M⁺]: 248.0869, found: 248.0862

5-Ethynyl-2-hydroxy-3-methoxybenzaldehyde



2-Hydroxy-3-methoxy-5-(2-(trimethylsilyl)ethynyl)benzaldehyde (701 mg, 2.83 mmol) was dissolved in dry THF (18 mL) and a freshly prepared solution of Bu₄NF in dry THF (5.34 mL, 5.34 mmol) was added. The reaction mixture was stirred at room temperature for 90 minutes and the solvent was evaporated under reduced pressure, diluted with CH₂Cl₂ and washed with water. The aqueous phase was extracted with CH₂Cl₂, the organic layers were combined, dried over anhydrous MgSO₄ and the solvent was evaporated under reduced pressure. The residue was dried in a vacuum desiccator over night and purified by gradient flash chromatography on silica gel (Hexane/EtOAc 5:1 to 2:1) to give 5-Ethynyl-2-hydroxy-

3-methoxybenzaldehyde as a fawn-yellow solid (470 mg, 94 %). $R_f = 0.27$ (Hexane/EtOAc 5:1)

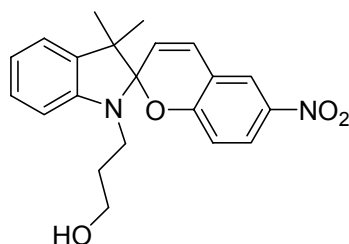
^1H NMR (300 MHz, CDCl_3): $\delta = 11.26$ (s, 1 H), 9.88 (s, 1 H), 7.37 (d, 1 H, $J = 1.8$ Hz), 7.18 (d, 1 H, $J = 1.6$ Hz), 3.93 (s, 3 H), 3.05 (s, 1H) - CI-MS (NH_3): m/z (%): 177.2 (12) $[\text{MH}^+]$, 194.2 (100) $[\text{M}+\text{NH}_4^+]$

General procedure A for synthesis of 3-propanol-spirobenzopyrans:

A Schlenk flask was charged with a deoxygenated 0.06 M solution of 3,4,10,10a-Tetrahydro-10,10,10a-trimethyl-2*H*-[1,3]oxazino[3,2-*a*]indole in freshly distilled EtOH. Following, the salicylaldehyde (1.0 eq.) was added under nitrogen atmosphere and the reaction mixture was sonicated at 35 kHz. The progress of the reaction was monitored by TLC until the starting materials disappeared or spot intensity of the product remained constant in successive controls. After completion of the reaction, EtOH was removed under reduced pressure, the residue was taken up in CH_2Cl_2 and dried over anhydrous Na_2SO_4 . The solution was filtered and the solvent was evaporated under reduced pressure. The residue was dried in vacuo and the crude product was purified by flash chromatography on silica gel.

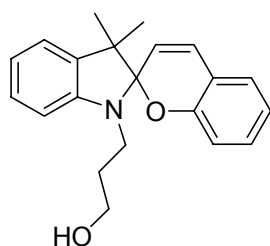
General procedure B for synthesis of 3-propanol-spirobenzopyrans:

A flask was capped with rubber septa and flushed with nitrogen for ten minutes. The flask was charged with a deoxygenated 0.1 M solution of 3,4,10,10a-Tetrahydro-10,10,10a-trimethyl-2*H*-[1,3]oxazino[3,2-*a*]indole in dry EtOH. The salicylaldehyde (1.0 eq.) was added under nitrogen atmosphere and the mixture was sonicated at 35 kHz. Progress of the reaction was monitored by TLC until the starting materials disappeared or spot intensity of the product remained constant in successive controls. Following, EtOH was removed under reduced pressure, and the residue taken up in CH_2Cl_2 and washed with water. The organic layer was dried over anhydrous Na_2SO_4 , the solvent was removed under reduced pressure, dried in vacuo and the crude product was purified by flash chromatography on silica gel.

3',3'-Dimethyl-6-nitro-Spiro[2H-1-benzopyran-2,2'-[2H]indole]-1'(3'H)-propanol

The compound was prepared following general procedure B and isolated as a pale purple solid. Ultrasonic irradiation time: 60 min. Yield: 84 %. Eluent for flash chromatography: Hexane/EtOAc 1:1 R_f = 0.43 (Hexane/EtOAc 1:1)

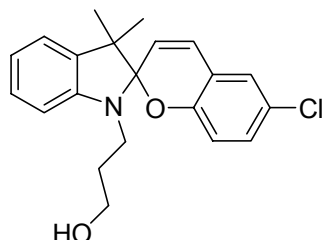
^1H NMR (600 MHz, CDCl_3): δ = 8.00 (td, 2 H, J = 2.7, 7.9 Hz), 7.19 (dt, 1 H, J = 1.2, 7.7 Hz), 7.09 (dd, 1 H, J = 0.8, 7.2 Hz), 6.91 (d, 1 H, J = 10.3 Hz), 6.88 (dt, 1 H, J = 0.8, 7.5 Hz), 6.75 (d, 1 H, J = 8.9 Hz), 6.65 (d, 1 H, J = 7.8 Hz), 5.88 (d, 1 H, J = 10.4 Hz), 3.71 (t, 2 H, J = 6.0 Hz, CH_2 -propyl), 3.40-3.34 (m, 1 H, CH_2 -propyl), 3.29-3.23 (m, 1 H, CH_2 -propyl), 1.98-1.90 (m, 1 H, CH_2 -propyl), 1.84-1.77 (m, 1 H, CH_2 -propyl), 1.29 (s, 3 H, CH_3), 1.19 (s, 3 H, CH_3) - ^{13}C NMR (150 MHz, CDCl_3): δ = 159.6 ($\text{C}_{\text{quat.}}$), 147.0 ($\text{C}_{\text{quat.}}$), 141.0 ($\text{C}_{\text{quat.}}$), 136.0 ($\text{C}_{\text{quat.}}$), 128.2 (+, CH), 127.8 (+, CH), 125.9 (+, CH), 122.7 (+, CH), 121.8 (+, CH), 121.7 (+, CH), 119.6 (+, CH), 118.5 ($\text{C}_{\text{quat.}}$), 115.5 (+, CH), 106.9 (+, CH), 106.8 ($\text{C}_{\text{quat.}}$), 60.7 (CH_2 -propyl), 52.6 ($\text{C}_{\text{quat.}}$), 40.7 (CH_2 -propyl), 31.6 (CH_2 -propyl), 25.9 (+, CH_3), 19.9 (+, CH_3) - IR (neat): ν [cm^{-1}] = 2970, 2359, 1738, 1479 - MS (ESI): m/z (%): 367.0 (100) [MH^+] - HRMS (EI-MS) calcd. for $\text{C}_{21}\text{H}_{22}\text{N}_2\text{O}_4$ [M^+]: 366.1580, found: 366.1572

3',3'-Dimethyl-Spiro[2H-1-benzopyran-2,2'-[2H]indole]-1'(3'H)-propanol

The compound was prepared following general procedure A and isolated as glistening blue foam. Ultrasonic irradiation time: 60 min. Yield: 87 %. Eluent for flash chromatography: Hexane/EtOAc 3:1 R_f = 0.20

^1H NMR (300 MHz, CDCl_3): δ = 7.17 (dt, 1 H, J = 1.3, 7.7 Hz), 7.13-7.02 (m, 3 H), 6.89-6.79 (m, 3 H), 6.70 (d, 1 H, J = 8.1 Hz), 6.62 (d, 1 H, J = 7.7 Hz), 5.68 (t, 1 H, J = 12.5 Hz), 3.71 (t, 2 H, J = 6.0 Hz), 3.45-3.33 (m, 1 H), 3.29-3.17 (m, 1 H), 2.02-1.73 (m, 2 H), 1.31 (s, 3 H), 1.17 (s, 3 H) - ^{13}C NMR (75 MHz, CDCl_3): δ = 154.2 ($\text{C}_{\text{quat.}}$), 147.5 ($\text{C}_{\text{quat.}}$), 136.7 ($\text{C}_{\text{quat.}}$), 129.8 (+, CH), 129.4 (+, CH), 127.6 (+, CH), 126.8 (+, CH), 121.7 (+, CH), 120.1 (+, CH), 119.6 (+, CH), 119.1 (+, CH), 118.5 ($\text{C}_{\text{quat.}}$), 115.1 (+, CH), 106.6 (+, CH), 104.6 ($\text{C}_{\text{quat.}}$), 61.1 (-, CH_2), 52.0 ($\text{C}_{\text{quat.}}$), 41.0 (-, CH_2), 31.7 (-, CH_2), 25.8 (+, CH_3), 20.3 (+, CH_3) - IR (neat): ν [cm^{-1}] = 2970, 2359, 1738, 1481, 1366 - MS (CI, 70 eV): m/z (%): 322.2 (100) [MH^+] - HRMS (EI-MS) calcd. for $\text{C}_{21}\text{H}_{23}\text{NO}_2$ [M^+]: 321.1729, found: 321.1726

3',3'-Dimethyl-6-chloro-Spiro[2H-1-benzopyran-2,2'-[2H]indole]-1'(3'H)-propanol

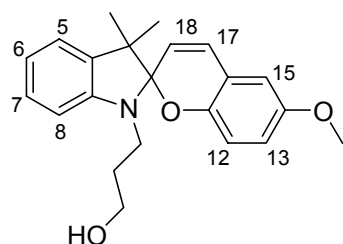


The compound was prepared following general procedure A and isolated as blue foam. Ultrasonic irradiation time: 60 min. Yield: 78 %. Eluent for flash chromatography: Hexane/EtOAc 3:1 R_f = 0.25

^1H NMR (300 MHz, CDCl_3): δ = 7.17 (t, 1 H, J = 7.6 Hz), 7.11-6.97 (m, 3 H), 6.90-6.74 (m, 2 H), 6.68-6.57 (m, 2 H), 5.76 (d, 1 H, J = 10.3 Hz), 3.70 (t, 2 H, J = 5.9 Hz), 3.43-3.30 (m, 1 H), 3.29-3.15 (m, 1 H), 2.01-1.74 (m, 2 H), 1.28 (s, 3 H), 1.16 (s, 3 H) - ^{13}C NMR (75 MHz, CDCl_3): δ = 152.7 ($\text{C}_{\text{quat.}}$), 147.4 ($\text{C}_{\text{quat.}}$), 136.4 ($\text{C}_{\text{quat.}}$), 129.4 (+, CH), 128.5 (+, CH), 127.7 (+, CH), 126.3 (+, CH), 124.7 ($\text{C}_{\text{quat.}}$), 121.7 (+, CH), 121.0 (+, CH), 119.8 ($\text{C}_{\text{quat.}}$), 119.2 (+, CH), 116.4 (+, CH), 106.7 (+, CH), 105.0 ($\text{C}_{\text{quat.}}$), 61.0 (-, CH_2), 52.2 ($\text{C}_{\text{quat.}}$), 40.9 (-, CH_2), 31.7 (-, CH_2), 25.8 (+, CH_3), 20.2 (+, CH_3) - IR (neat): ν [cm^{-1}] = 2970, 2363, 1738, 1476,

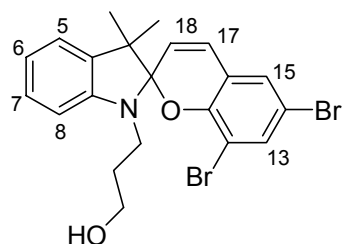
1371, 1217 - MS (CI, 70 eV): m/z (%): 356.2 (100) $[MH^+]$ - HRMS (EI-MS) calcd. for $C_{21}H_{22}ClNO_2$ $[M^+]$: 355.1339, found: 355.1336

3',3'-Dimethyl-6-methoxy-Spiro[2H-1-benzopyran-2,2'-[2H]indole]-1'(3'H)-propanol



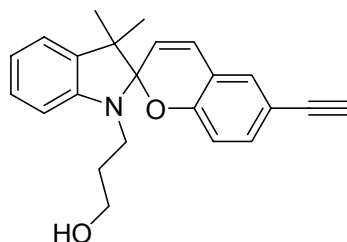
The compound was prepared following general procedure A and isolated as pale blue foam. Ultrasonic irradiation time: 60 min. Yield: 54 %. Eluent for flash chromatography: Hexane/EtOAc R_f = 0.23

1H NMR (600 MHz, $CDCl_3$): δ = 7.21 (ddd, 1 H, J = 1.3, 7.5, 7.8 Hz, C7-CH), 7.13 (ddd, 1 H, J = 0.5, 1.3, 7.2 Hz, C5-CH), 6.89 (ddd, 1 H, J = 1.0, 7.2, 7.5, C6-CH), 6.84 (d, 1 H, J = 10.2 Hz, C17-CH), 6.72 (dd, 1 H, J = 2.9, 8.8 Hz, C13-CH), 6.69 (d, 1 H, J = 8.8 Hz, C12-CH), 6.67 (d, 1 H, J = 2.9 Hz, C15-CH), 6.66 (dd, 1 H, J = 1.0, 7.8 Hz, C8-CH), 5.77 (d, 1 H, 10.2 Hz, C18-CH), 3.79 (s, 3 H, OCH_3), 3.73-3.67 (m, 2 H, CH_2 -propyl), 3.46-3.40 (m, 1 H, CH_2 -propyl), 3.30-3.24 (m, 1 H, CH_2 -propyl), 2.01-1.92 (m, 1 H, CH_2 -propyl), 1.89-1.79 (m, 1 H, CH_2 -propyl), 1.37 (s, 3 H, C3-Me), 1.23 (s, 3 H, C3-Me) - ^{13}C NMR (75 MHz, $CDCl_3$): δ = 153.0 ($C_{quat.}$), 148.1 ($C_{quat.}$), 147.4 ($C_{quat.}$), 136.5 ($C_{quat.}$), 129.1 (C17), 127.4 (C7), 121.5 (C5), 120.4 (C18), 118.8 (C6), 118.7 ($C_{quat.}$), 115.4 (C12), 115.2 (C13), 111.4 (C15), 106.4 (C8), 104.2 ($C_{quat.}$), 60.7 (-, CH_2 -propyl), 55.6 (OCH_3), 51.7 ($C_{quat.}$), 40.7 (-, CH_2 -propyl), 31.5 (-, CH_2 -propyl), 25.6 (+, CH_3), 20.2 (+, CH_3) - IR (neat): ν [cm^{-1}] = 2970, 2357, 1738, 1481, 1366 - MS (CI, 70 eV): m/z (%): 352.2 (100) $[MH^+]$ - HRMS (EI-MS) calcd. for $C_{22}H_{25}NO_3$ $[M^+]$: 351.1834, found: 351.1833

3',3'-Dimethyl-6,8-dibromo-Spiro[2H-1-benzopyran-2,2'-[2H]indole]-1'(3'H)-propanol


The compound was prepared following general procedure A and isolated as dark blue foam. Ultrasonic irradiation time: 60 min. Yield: 93 %. Eluent for flash chromatography: Hexane/EtOAc 2:1 R_f = 0.33

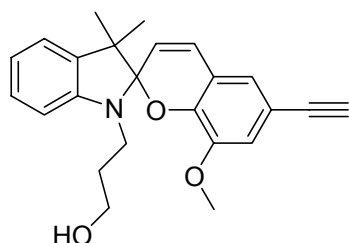
^1H NMR (400 MHz, CDCl_3): δ = 7.45 (d, 1 H, J = 2.3 Hz, C13-CH), 7.15 (ddd, 1 H, J = 1.3, 7.5, 7.8 Hz, C7-CH), 7.12 (d, 1 H, J = 2.3 Hz, C15-CH), 7.06 (ddd, 1 H, J = 0.5, 1.3, 7.3 Hz, C5-CH), 6.84 (ddd, 1 H, J = 0.9, 7.3, 7.5 Hz, C6-CH), 6.73 (d, 1 H, J = 10.3 Hz, C17-CH), 6.61 (dd, 1 H, J = 0.9, 7.8 Hz, C8-CH), 5.76 (d, 1 H, J = 10.3 Hz, C18-CH), 3.74-3.67 (m, 2 H, CH_2 -propyl), 3.41-3.31 (m, 1 H, CH_2 -propyl), 3.29-3.20 (m, 1 H, CH_2 -propyl), 1.98-1.87 (m, 1 H, CH_2 -propyl), 1.84-1.73 (m, 1 H, CH_2 -propyl), 1.30 (s, 3 H), 1.17 (s, 3 H) - ^{13}C NMR (100 MHz, CDCl_3): δ = 149.8 ($\text{C}_{\text{quat.}}$), 147.0 ($\text{C}_{\text{quat.}}$), 136.1 ($\text{C}_{\text{quat.}}$), 134.8 (C13), 128.3 (C15), 127.8 (C17), 127.6 (C7), 122.2 (C18), 121.6 (C5), 121.3 ($\text{C}_{\text{quat.}}$), 119.2 (C6), 111.8 ($\text{C}_{\text{quat.}}$), 110.1 ($\text{C}_{\text{quat.}}$), 106.6 (C8), 106.4 ($\text{C}_{\text{quat.}}$), 60.9 (-, CH_2 -propyl), 52.2 ($\text{C}_{\text{quat.}}$), 40.6 (-, CH_2 -propyl), 31.5 (-, CH_2 -propyl), 25.5 (+, CH_3), 20.5 (+, CH_3) - IR (neat): ν [cm^{-1}] = 2970, 2357, 1738, 1445, 1366, 1217 - MS (CI, 70 eV): m/z (%) = 478.0 (59) [MH^+], 480.1 (100), 482.1 (55)

3',3'-Dimethyl-6-ethynyl-Spiro[2H-1-benzopyran-2,2'-[2H]indole]-1'(3'H)-propanol


The compound was prepared following general procedure A and isolated as pale blue foam. Ultrasonic irradiation time: 120 min. Yield: 68 %. Eluent for flash chromatography: Hexane/EtOAc 3:1 R_f = 0.20

^1H NMR (300 MHz, CDCl_3): δ = 7.28-7.16 (m, 3 H), 7.13-7.07 (m, 1 H), 6.92-6.80 (m, 2 H), 6.70-6.62 (m, 2 H), 5.81-5.74 (d, 1 H), 3.77-3.69 (m, 2 H), 3.45-3.33 (m, 1 H), 3.31-3.20 (m, 1 H), 3.00 (s, 1 H), 2.02-1.78 (m, 2 H), 1.31 (s, 3 H), 1.19 (s, 3 H) - ^{13}C NMR (75 MHz, CDCl_3): δ = 154.7 ($\text{C}_{\text{quat.}}$), 147.4 ($\text{C}_{\text{quat.}}$), 136.4 ($\text{C}_{\text{quat.}}$), 133.8 (+, CH), 130.6 (+, CH), 128.8 (+, CH), 127.6 (+, CH), 121.7 (+, CH), 120.5 (+, CH), 119.3 (+, CH), 118.6 ($\text{C}_{\text{quat.}}$), 115.3 (+, CH), 106.7 (+, CH), 105.2 ($\text{C}_{\text{quat.}}$), 83.5 ($\text{C}_{\text{quat.}}$), 75.6 (+, CH), 61.0 (-, CH_2), 52.2 ($\text{C}_{\text{quat.}}$), 40.9 (-, CH_2), 31.7 (-, CH_2), 25.9 (+, CH_3), 21.1 ($\text{C}_{\text{quat.}}$), 20.1 (+, CH_3) - IR (neat): ν [cm^{-1}] = 3449, 3281, 2970, 2357, 1740, 1481, 1373 - MS (ESI): m/z (%): 346.0 (100) [MH^+] - HRMS (EI-MS) calcd. for $\text{C}_{23}\text{H}_{23}\text{NO}_2$ [M^+]: 345.1729, found: 345.1730

3',3'-Dimethyl-6-ethynyl-8-methoxy-Spiro[2H-1-benzopyran-2,2'-[2H]indole]-1'(3'H)-propanol

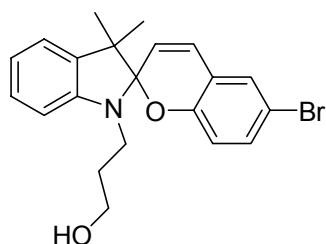


The compound was prepared following general procedure A and isolated as glistening turquoise foam. Ultrasonic irradiation time: 100 min. Yield: 75 %. Eluent for gradient flash chromatography: Hexane/EtOAc 3:1 to 2:1 R_f = 0.15 (Hexane/EtOAc 3:1)

^1H NMR (600 MHz, CDCl_3): δ = 7.17-7.03 (m, 4 H), 6.82 (t, 1 H, J = 7.3 Hz), 6.75 (d, 1 H, J = 10.2 Hz), 6.59 (d, 1 H, J = 7.7 Hz), 5.76 (d, 1 H, J = 10.2 Hz), 3.91 (s, 1 H), 3.68 (s, 3 H), 3.75-3.69 (m, 2 H), 3.41-3.34 (m, 1 H), 3.29-3.24 (m, 1 H), 1.94-1.87 (m, 1 H), 1.85-1.77 (m, 1 H), 1.29 (s, 3 H), 1.16 (s, 3 H) ^{13}C NMR (150 MHz, CDCl_3): δ = 147.2, 146.9, 144.4, 136.4, 128.4, 127.5, 123.4, 121.7, 120.9, 119.2, 118.9, 116.9, 113.0, 106.5, 105.2, 83.6, 75.4, 60.8, 56.3, 51.9, 40.5, 31.5, 25.6, 20.4 - IR (neat): ν [cm^{-1}] = 3287, 2959, 2357, 1479, 1458 - MS

(ESI): m/z (%): 376.0 $[MH^+]$ - HRMS (EI-MS) calcd. for $C_{24}H_{25}NO_3$ $[M^+]$: 375.1834, found: 375.1840

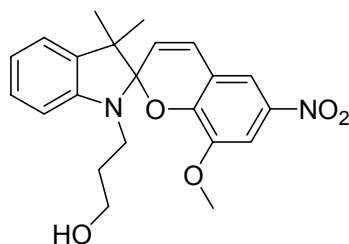
3',3'-Dimethyl-6-bromo-Spiro[2H-1-benzopyran-2,2'-[2H]indole]-1'(3'H)-propanol



The compound was prepared following general procedure B and isolated as glistening grey-blue foam. Ultrasonic irradiation time: 70 min. Yield: 52 %. Eluent for flash chromatography: Hexane/EtOAc 2:1 R_f = 0.47

1H NMR (400 MHz, $CDCl_3$): δ = 7.20-7.14 (m, 3 H), 7.09-7.05 (d, 1 H), 6.87-6.82 (dt, 1 H), 6.79-6.75 (d, 1 H), 6.64-6.56 (m, 2 H), 5.77-5.73 (d, 1 H), 3.72-3.67 (m, 2 H), 3.40-3.30 (m, 1 H), 3.27-3.18 (m, 1 H), 1.97-1.89 (m, 1 H), 1.84-1.76 (m, 1 H), 1.29 (s, 3 H), 1.16 (s, 3 H) - ^{13}C NMR (100 MHz, $CDCl_3$): δ = 153.2 ($C_{quat.}$), 147.3 ($C_{quat.}$), 136.4 ($C_{quat.}$), 132.3 (+, CH), 129.1 (+, CH), 128.3 (+, CH), 127.6 (+, CH), 121.7 (+, CH), 120.9 (+, CH), 120.4 ($C_{quat.}$), 119.2 (+, CH), 116.9 (+, CH), 111.9 ($C_{quat.}$), 106.7 (+, CH), 105.0 ($C_{quat.}$), 60.9 (-, CH_2), 52.2 ($C_{quat.}$), 40.8 (-, CH_2), 31.7 (-, CH_2), 25.8 (+, CH_3), 20.1 (+, CH_3) - MS (ESI): m/z (%) = 401.9 (100) $[MH^+]$

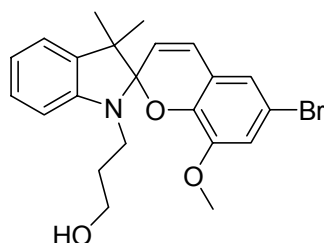
3',3'-Dimethyl-8-methoxy-6-nitro-Spiro[2H-1-benzopyran-2,2'-[2H]indole]-1'(3'H)-propanol



The compound was prepared following general procedure A and isolated as lustrous blue foam. Ultrasonic irradiation time: 75 min. Yield: 83 %. Eluent for flash chromatography: Hexane/EtOAc 1:1 R_f = 0.49

^1H NMR (600 MHz, CDCl_3): δ = 7.69-7.67 (m, 1 H), 7.62 (d, 1 H, J = 2.6 Hz), 7.18-7.15 (m, 1 H), 7.07 (d, 1 H, J = 7.3 Hz), 6.87-6.84 (m, 2 H), 6.62 (d, 1 H, J = 7.8 Hz), 5.84 (d, 1 H, J = 10.3 Hz), 3.76 (s, 3 H), 3.71-3.68 (m, 2 H), 3.41-3.35 (m, 1 H), 3.32-3.26 (m, 1 H), 2.95-2.88 (m, 1 H), 1.84-1.79 (m, 1 H), 1.28 (s, 3 H), 1.18 (s, 3 H) – ^{13}C NMR (150 MHz, CDCl_3): δ = 149.3, 147.3, 147.0, 140.4, 136.0, 128.0, 127.6, 122.0, 121.7, 119.3, 118.3, 116.1, 115.4, 107.9, 106.7, 60.6, 56.3, 52.5, 40.5, 31.6, 25.8, 20.1 - IR (neat): ν [cm^{-1}] = 3016, 2970, 2359, 1738, 1366, 1229 - MS (EI, 70 eV): m/z (%): 397.1 (100) [MH^+] - HRMS (EI-MS) calcd. for $\text{C}_{22}\text{H}_{24}\text{N}_2\text{O}_5$ [M^+]: 396.1685, found: 396.1685

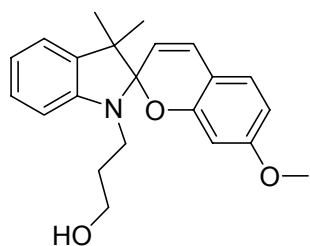
3',3'-Dimethyl-6-bromo-8-methoxy-Spiro[2H-1-benzopyran-2,2'-[2H]indole]-1'(3'H)-propanol



The compound was prepared following general procedure A and isolated as pale blue-green foam. Ultrasonic irradiation time: 60 min. Yield: 76 %. Eluent for gradient flash chromatography: Hexane/EtOAc 3:1 to 1:1 R_f = 0.22 (Hexane/EtOAc 3:1)

^1H NMR (600 MHz, CDCl_3): δ = 7.14 (t, 1 H, J = 7.6 Hz), 7.05 (dd, 1 H, J = 1.0, 7.3 Hz), 6.84 (d, 1H, J = 2.2 Hz), 6.83-6.81 (m, 2 H), 6.71 (d, 1 H, J = 10.2 Hz), 6.58 (d, 1 H, J = 7.7 Hz), 5.71 (d, 1 H, J = 10.2 Hz), 3.76-3.68 (m, 2 H), 3.67 (s, 3 H), 3.39-3.33 (m, 1 H), 3.28-3.23 (m, 1 H), 1.93-1.86 (m, 1 H), 1.84-1.77 (m, 1 H), 1.29 (s, 3 H), 1.16 (s, 3 H) – ^{13}C NMR (75 MHz, CDCl_3): δ = 148.1, 147.2, 136.4, 127.9, 127.5, 121.7, 121.5, 120.6, 118.9, 116.5, 111.4, 108.8, 106.5, 104.9, 101.2, 60.8, 56.5, 51.9, 40.6, 31.6, 25.6, 20.6 - IR (neat): ν [cm^{-1}] = 2970, 2259, 1738, 1366, 1217 - HRMS (EI-MS) calcd. for $\text{C}_{22}\text{H}_{24}\text{BrNO}_3$ [M^+]: 429.0940, found: 429.0930

3',3'-Dimethyl-7-methoxy-Spiro[2H-1-benzopyran-2,2'-[2H]indole]-1'(3'H)-propanol

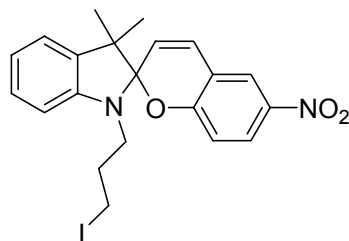


The compound was prepared following general procedure A and isolated as pale pink solid. Ultrasonic irradiation time: 60 min. Yield: 82 %. Eluent for gradient flash chromatography: $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 20:1 to 10:1 R_f = 0.28 ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 20:1)

^1H NMR (300 MHz, CDCl_3): δ = 7.17 (dt, 1 H, J = 1.2, 7.6 Hz), 7.09 (dd, 1 H, J = 0.8, 7.1 Hz), 6.94 (d, 1 H, J = 8.3 Hz), 6.85 (dt, 1 H, J = 0.9, 7.5 Hz), 6.80 (d, 1 H, J = 10.0 Hz), 6.62 (d, 1 H, J = 7.7 Hz), 6.39 (dd, 1 H, J = 2.5, 8.3 Hz), 6.29 (d, 1 H, J = 2.5 Hz), 5.56 (d, 1 H, J = 10.2 Hz), 3.81-3.73 (m, 1 H), 3.71 (s, 3 H), 3.69-3.62 (m, 2 H), 3.45-3.32 (m, 1 H), 3.29-3.18 (m, 1 H), 1.98-1.88 (m, 1 H), 1.85-1.73 (m, 1 H), 1.31 (s, 3 H), 1.17 (s, 3 H) - ^{13}C NMR (75 MHz, CDCl_3): δ = 161.1, 155.5, 147.6, 136.8, 129.2, 127.6, 127.5, 121.7, 119.2, 119.1, 116.3, 111.9, 106.7, 105.1, 100.2, 61.2, 55.3, 51.8, 40.9, 31.7, 25.9, 20.1 - IR (neat): ν [cm^{-1}] = 2970, 2359, 1740, 1366, 1217 - MS (ESI): m/z (%) = 352.0 (100) [MH^+]

General procedure C for synthesis of 3-Iodopropyl-spirobenzopyrans:

A flask was capped with rubber septa and flushed with nitrogen for 10 minutes. The flask was charged with freshly prepared 1-(3-Iodopropyl)-3,3-dimethyl-2-methyleneindoline and dry, degassed EtOH was added to obtain a 0.1 M solution. The solution was deoxygenated, following, salicylaldehyde (1.0 eq.) was added under nitrogen atmosphere and the mixture was sonicated at 35 kHz. Progress of the reaction was monitored by TLC until the starting materials disappeared or spot intensity of the product remained constant in successive controls. Following, the solvent was removed under reduced pressure, and the remaining residue taken up in CH₂Cl₂ and washed with water. The organic layer was dried over anhydrous MgSO₄, the solvent was removed under reduced pressure and the raw product was dried in vacuo, followed by purification using flash chromatography on silica gel.

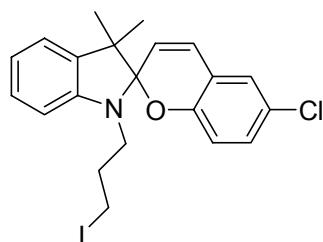
1'-(3-Iodopropyl)-1',3'-dihydro-3',3'-dimethyl-6-nitro-Spiro[2H-1-benzopyran-2,2'[2H]indole]

The compound was prepared following general procedure C and isolated as golden foam. Ultrasonic irradiation time: 60 min. Yield: 94 %. Eluent for flash chromatography: CH₂Cl₂ R_f = 0.83

¹H NMR (400 MHz, CDCl₃): δ = 8.06-8.01 (m, 2 H, H-Ar), 7.23 (dt, 1 H, J = 1.3, 7.7 Hz, H-Ar), 7.14 (dd, 1 H, J = 0.9, 7.3 Hz, H-Ar), 6.99 (d, 1 H, J = 10.2 Hz, H-Ar), 6.93 (dt, 1 H, J = 0.8, 7.5 Hz, H-Ar), 6.76 (d, 1 H, J = 8.5 Hz, H-Ar), 6.69 (d, 1 H, J = 7.7 Hz, H-Ar), 5.93 (d, 1 H, J = 10.3 Hz, H-Ar), 3.41-3.17 (m, 4 H, 2 x CH₂), 2.34-2.21 (m, 1 H, CH₂-propyl), 2.17-2.06 (m, 1 H, CH₂-propyl), 1.34 (s, 3 H, CH₃), 1.23 (s, 3 H, CH₃) - ¹³C NMR (100 MHz, CDCl₃): δ = 159.2 (C_{quat.}), 146.6 (C_{quat.}), 140.8 (C_{quat.}), 135.8 (C_{quat.}), 128.4 (+, CH), 127.6 (+, CH), 125.7 (+, CH), 122.6 (+, CH), 121.6 (+, CH), 121.6 (+, CH), 119.6 (+, CH), 118.3 (C_{quat.}), 115.3 (+, CH), 106.6 (+, CH), 106.4 (C_{quat.}), 52.4 (C_{quat.}), 43.9 (-, CH₂), 32.3 (-, CH₂),

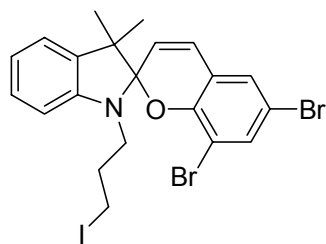
25.8 (+, CH₃), 19.7 (+, CH₃), 3.3 (-, CH₂) - MS (ESI): m/z (%): 477.1 (100) [MH⁺] - HRMS (EI-MS) calcd. for C₂₁H₂₁N₂O₃I [M⁺]: 476.0597, found: 476.0598

1'-(3-Iodopropyl)-1',3'-dihydro-3',3'-dimethyl-6-chloro-Spiro[2H-1-benzopyran-2,2'-[2H]indole]



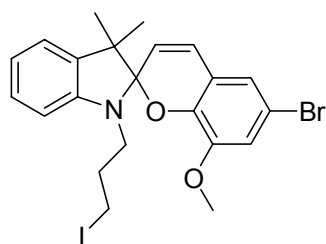
The compound was prepared following general procedure C and isolated as orange foam. Ultrasonic irradiation time: 60 min. Yield: 74 %. Eluent for flash chromatography: CH₂Cl₂ R_f = 0.87

¹H NMR (300 MHz, CDCl₃): δ = 7.18 (dt, 1 H, J = 1.3, 7.7 Hz), 7.10-7.02 (m, 3 H), 6.86 (t, 1 H, J = 7.4 Hz), 6.80 (d, 1 H, J = 10.2 Hz), 6.62 (t, 2 H, J = 7.9 Hz), 5.74 (d, 1 H, J = 10.3 Hz), 3.38-3.27 (m, 1 H), 3.25-3.11 (m, 3 H), 2.29-2.16 (m, 1 H), 2.14-1.99 (m, 1 H), 1.29 (s, 3 H), 1.16 (s, 3 H) - ¹³C NMR (75 MHz, CDCl₃): δ = 152.6 (C_{quat.}), 147.2 (C_{quat.}), 136.4 (C_{quat.}), 129.5 (+, CH), 128.8 (+, CH), 127.6 (+, CH), 126.3 (+, CH), 124.8 (C_{quat.}), 121.8 (+, CH), 120.9 (+, CH), 119.8 (C_{quat.}), 119.3 (+, CH), 116.4 (+, CH), 106.6 (+, CH), 104.8 (C_{quat.}), 52.2 (C_{quat.}), 44.1 (-, CH₂), 32.7 (-, CH₂), 25.9 (+, CH₃), 20.1 (+, CH₃), 3.6 (-, CH₂) - IR (neat): ν [cm⁻¹] = 2961, 2357, 1606, 1476 - MS (ESI): m/z (%): 465.9 (100) [MH⁺] - HRMS (EI-MS) calcd. for C₂₁H₂₁ClNOI [M⁺]: 465.0356, found: 465.0352

1'-(3-Iodopropyl)-1',3'-dihydro-3',3'-dimethyl-6,8-dibromo -Spiro[2H-1-benzopyran-2,2'-[2H]indole]

The compound was prepared following general procedure C and isolated as turquoise foam. Ultrasonic irradiation time: 60 min. Yield: 83 %. Eluent for flash chromatography: CH₂Cl₂ R_f = 0.90

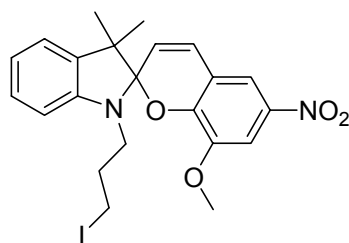
¹H NMR (300 MHz, CDCl₃): δ = 7.46 (d, 1 H, J = 2.3 Hz), 7.17 (m, 2 H), 7.08 (d, 1 H, J = 6.3 Hz), 6.86 (t, 1 H, J = 7.4 Hz), 6.77 (d, 1 H, J = 10.2 Hz), 6.61 (d, 1 H, J = 7.7 Hz), 5.76 (d, 1 H, J = 10.2 Hz), 3.40-3.28 (m, 1 H), 3.27-3.12 (m, 3 H), 2.31-2.15 (m, 1 H), 2.13-1.98 (m, 1 H), 1.30 (s, 3 H), 1.17 (s, 3 H) - ¹³C NMR (75 MHz, CDCl₃): δ = 149.67 (C_{quat.}), 146.8 (C_{quat.}), 136.1 (C_{quat.}), 134.9 (+, CH), 128.4 (+, CH), 128.1 (+, CH), 127.6 (+, CH), 122.1 (+, CH), 121.8 (+, CH), 121.3 (C_{quat.}), 119.4 (+, CH), 111.9 (C_{quat.}), 110.2 (C_{quat.}), 106.6 (+, CH), 106.2 (C_{quat.}), 52.3 (C_{quat.}), 44.0 (-, CH₂), 32.3 (-, CH₂), 25.7 (+, CH₃), 20.5 (+, CH₃), 3.9 (-, CH₂) - IR (neat): ν [cm⁻¹] = 2960, 2357, 1606, 1481, 1444, 1364 - MS (ESI): m/z (%): 587.8 (100) [MH⁺] - HRMS (EI-MS) calcd. for C₂₁H₂₀Br₂INO [M⁺]: 586.8957, found: 586.8961

1'-(3-Iodopropyl)-1',3'-dihydro-3',3'-dimethyl-6-bromo-8-methoxy-Spiro[2H-1-benzopyran-2,2'-[2H]indole]

The compound was prepared following general procedure C and isolated as dark orange crystals. Ultrasonic irradiation time: 50 min. Yield: 80 %. Eluent for flash chromatography: CH₂Cl₂ R_f = 0.63

¹H NMR (300 MHz, CDCl₃): δ = 7.16 (dt, 1 H, J = 1.2, 7.6 Hz), 7.06 (dd, 1 H, J = 0.9, 7.2 Hz), 6.87-6.80 (m, 3 H), 6.75 (d, 1 H, J = 10.2 Hz), 6.58 (d, 1 H, J = 7.7 Hz), 5.71 (d, 1 H, J = 10.2 Hz), 3.27-3.11 (s, 3 H), 3.43-3.30 (m, 1 H), 3.27-3.11 (m, 3 H), 2.27-2.00 (m, 2 H), 1.29 (s, 3 H), 1.15 (s, 3 H) - ¹³C NMR (75 MHz, CDCl₃): δ = 148.1 (C_{quat.}), 147.1 (C_{quat.}), 142.6 (C_{quat.}), 136.4 (C_{quat.}), 128.5 (+, CH), 127.5 (+, CH), 121.8 (+, CH), 121.5 (+, CH), 121.2 (+, CH), 120.5 (C_{quat.}), 119.0 (+, CH), 116.5 (+, CH), 111.4 (C_{quat.}), 106.4 (+, CH), 104.7 (C_{quat.}), 56.4 (+, CH₃), 52.2 (C_{quat.}), 44.0 (-, CH₂), 32.7 (-, CH₂), 26.0 (+, CH₃), 20.2 (+, CH₃), 3.8 (-, CH₂) - IR (neat): ν [cm⁻¹] = 2960, 2930, 2862, 1606, 1476, 1381 - MS (ESI): m/z (%): 539.8 (100) [MH⁺] - HRMS (EI-MS) calcd. for C₂₂H₂₃BrINO₂ [M⁺]: 538.9957, found: 538.9952

1'-(3-Iodopropyl)-1',3'-dihydro-3',3'-dimethyl-6-nitro-8-methoxy-Spiro[2H-1-benzopyran-2,2'-[2H]indole]

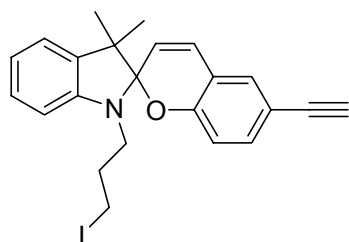


The compound was prepared following general procedure C and isolated as turquoise-blue foam. Ultrasonic irradiation time: 60 min. Yield: 68 %. Eluent for flash chromatography: CH₂Cl₂ R_f = 0.75

¹H NMR (300 MHz, CDCl₃): δ = 7.70 (d, 1 H, J = 2.5 Hz), 7.63 (d, 1 H, J = 2.5 Hz), 7.18 (dt, 1 H, J = 1.3, 7.7 Hz), 7.08 (dd, 1 H, J = 0.9, 7.3 Hz), 6.87 (m, 2 H), 6.61 (d, 1 H, J = 7.8 Hz), 5.83 (d, 1 H, J = 10.3 Hz), 3.79-3.74 (s, 3 H), 3.41-3.12 (m, 4 H), 2.28-2.01 (m, 2 H), 1.28 (s, 3 H), 1.17 (s, 3 H) - ¹³C NMR (75 MHz, CDCl₃): δ = 149.1 (C_{quat.}), 147.4 (C_{quat.}), 146.8 (C_{quat.}), 140.5 (C_{quat.}), 136.0 (C_{quat.}), 128.5 (+, CH), 127.6 (+, CH), 121.9 (+, CH), 121.8 (+, CH), 119.5 (+, CH), 118.2 (C_{quat.}), 115.4 (+, CH), 107.9 (+, CH), 106.7 (+, CH), 106.4 (C_{quat.}), 56.3 (+, CH₃), 52.6 (C_{quat.}), 43.9 (-, CH₂), 32.5 (-, CH₂), 26.1 (+, CH₃), 20.0 (+, CH₃), 3.4 (-, CH₂)

CH₂) - IR (neat): ν [cm⁻¹] = 2965, 1517, 1476, 1334 - MS (ESI): m/z (%): 506.9 (100) [MH⁺]
- HRMS (EI-MS) calcd. for C₂₂H₂₃IN₂O₄ [M⁺]: 506.0703, found: 506.0699

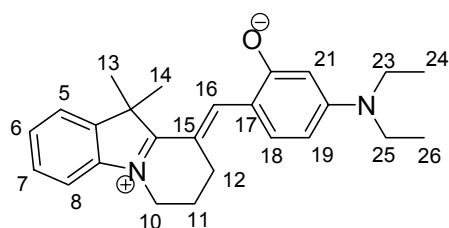
1'-(3-Iodopropyl)-1',3'-dihydro-3',3'-dimethyl-6-ethynyl-Spiro[2H-1-benzopyran-2,2'-[2H]indole]



The compound was prepared following general procedure C and isolated as yellow-orange crystals. Ultrasonic irradiation time: 65 min. Yield: 58 %. Eluent for gradient flash chromatography: CH₂Cl₂ to CH₂Cl₂/MeOH 30:1 R_f = 0.75 (CH₂Cl₂)

¹H NMR (300 MHz, CDCl₃): δ = 7.22 (m, 3 H), 7.10 (d, 1 H, J = 6.3 Hz), 6.87 (dd, 2 H, J = 8.8 Hz, J = 14.1 Hz), 6.64 (t, 2 H, J = 7.7 Hz), 5.74 (d, 1 H, J = 10.3 Hz), 3.40-3.30 (m, 1 H), 3.26-3.11 (m, 3 H), 2.99 (s, 1 H), 2.31-2.17 (m, 1 H), 2.15-2.02 (m, 1 H), 1.30 (s, 3 H), 1.18 (s, 3 H) - ¹³C NMR (75 MHz, CDCl₃): δ = 154.6 (C_{quat.}), 147.2 (C_{quat.}), 136.4 (C_{quat.}), 133.9 (+, CH), 130.7 (+, CH), 129.1 (+, CH), 127.7 (+, CH), 121.8 (+, CH), 120.4 (+, CH), 119.4 (+, CH), 118.6 (C_{quat.}), 115.4 (+, CH), 113.7 (C_{quat.}), 106.6 (+, CH), 105.1 (C_{quat.}), 83.5 (C_{quat.}), 75.7 (+, CH), 52.2 (C_{quat.}), 44.1 (-, CH₂), 32.7 (-, CH₂), 26.0 (+, CH₃), 20.1 (+, CH₃), 3.6 (-, CH₂) - MS (ESI): m/z (%): 455.9 (100) [MH⁺] - HRMS (EI-MS) calcd. for C₂₃H₂₂INO [M⁺]: 455.0746, found: 455.0745

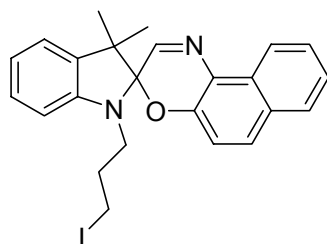
9-[[4-(dimethylamino)-2-phenolate]methylene]-7,8,9,10-tetrahydro-10,10-dimethyl-6H-Pyrido[1,2-a]indolium



A Schlenk flask was charged with dry EtOH (12 mL) and freshly prepared 1-(3-Iodopropyl)-3,3-dimethyl-2-methyleneindoline (372 mg, 1.14 mmol). The solution was deoxygenated and 5-(diethylamino)-2-hydroxybenzaldehyde (220 mg, 1.14 mmol) was added under nitrogen atmosphere. The reaction mixture was sonicated at 35 kHz for 60 minutes. Following, EtOH was removed under reduced pressure, the residue was dissolved in CH_2Cl_2 and dried over anhydrous Na_2SO_4 . The solution was filtered and the solvent was evaporated under reduced pressure. The residue was dried in vacuo to afford the crude product as glistening purple-green crystals. Purification by gradient flash chromatography on silica gel ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 19:1 to 12:1) afforded the product as a lustrous purple-green solid (87 %). $R_f = 0.30$ ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 20:1)

^1H NMR (600 MHz, CDCl_3): δ = 8.53 (s, 1H), 7.56 (d, 1 H, J = 9.5 Hz, C8-H), 7.43-7.39 (m, 2 H), 7.33 (t, 1 H, J = 7.1 Hz), 7.30-7.27 (m, 2 H), 4.15 (m, 2 H), 3.45 (q, 4 H, J = 7.1 Hz, C23/25), 2.98 (t, 2 H, J = 6.2 Hz), 2.24 (m, 2 H), 1.70 (s, 6 H, C13/14), 1.23 (t, 6 H, J = 7.1 Hz, C24/26) - ^{13}C NMR (150 MHz, CDCl_3): δ = 176.6 ($\text{C}_{\text{quat.}}$), 164.0 ($\text{C}_{\text{quat.}}$), 154.8 ($\text{C}_{\text{quat.}}$), 145.8 (CH, C16), 141.6 ($\text{C}_{\text{quat.}}$), 141.1 ($\text{C}_{\text{quat.}}$), 133.1 (CH), 128.7 (CH), 126.8 (CH), 122.3 (CH), 113.6 ($\text{C}_{\text{quat.}}$), 111.2 (CH), 106.9 (CH), 98.3 (CH), 50.6 ($\text{C}_{\text{quat.}}$), 45.3 (CH_3 , C24/26), 43.5 (C10), 28.5 ($\text{C}_{\text{quat.}}$), 24.9 CH_2 , C12), 20.3 (CH_2 , C11), 12.9 (CH_2 , C23/25) - MS (ESI): m/z (%): 375.0 (100) [MH^+]

1'-(3-Iodopropyl)-1',3'-dihydro-3',3'-dimethyl-spiro[3H]naphth[2,1-b][1,4]oxazine



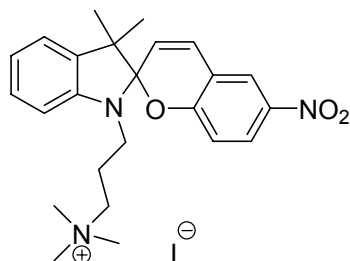
A Schlenk flask was charged with freshly prepared 1-(3-Iodopropyl)-3,3-dimethyl-2-methyleneindoline (348 mg, 1.06 mmol) and dry EtOH (12 mL) under N₂ atmosphere. The solution was degassed and 1-Nitroso-2-naphthol (203 mg, 1.17 mmol) was added under a slow flow of N₂. The mixture was sonicated for 2 hours at 35 kHz, then the solvent was removed under reduced pressure, the residue was dried in vacuo and the crude product was purified by gradient flash chromatography on silica gel (CH₂Cl₂ to CH₂Cl₂/MeOH 200:1) to yield 1'-(3-Iodopropyl)-1',3'-dihydro-3',3'-dimethyl-spiro[3H]naphth[2,1-b][1,4]oxazine as a yellow powder (126 mg, 25 %). R_f = 0.60 (CH₂Cl₂)

¹H NMR (600 MHz, CDCl₃): δ = 8.55 (d, 1 H, J = 8.4 Hz, H-Ar), 7.78-7.72 (m, 2 H, H-Ar), 7.68 (d, 1 H, J = 8.9 Hz, H-Ar), 7.58 (ddd, 1 H, J = 1.1, 6.9, 8.3 Hz, H-Ar), 7.40 (ddd, 1 H, J = 1.2, 6.9, 8.1 Hz, H-Ar), 7.22 (dt, 1 H, J = 1.2, 7.7 Hz, H-Ar), 7.09 (dd, 1 H, J = 1.0, 7.3 Hz, H-Ar), 7.00 (d, 1 H, J = 8.9 Hz, H-Ar), 6.90 (dt, 1 H, J = 0.7, 7.4 Hz, H-Ar), 6.67 (d, 1 H, J = 7.8 Hz, H-Ar), 3.42-3.22 (m, 2 H, CH₂-propyl), 3.20-3.09 (m, 2 H, CH₂-propyl), 2.30-2.08 (m, 2 H, CH₂-propyl), 1.35 (s, 3 H, CH₃), 1.34 (s, 3 H, CH₃) - ¹³C NMR (150 MHz, CDCl₃): δ = 151.0 (CH), 146.7 (C_{quat.}), 143.7 (C_{quat.}), 135.6 (C_{quat.}), 130.8 (C_{quat.}), 130.4 (CH), 129.3 (C_{quat.}), 128.0 (CH), 127.8 (CH), 127.2 (CH), 124.2 (CH), 122.7 (C_{quat.}), 121.8 (CH), 121.5 (CH), 119.8 (CH), 116.8 (CH), 106.9 (CH), 98.9 (C_{quat.}), 52.1 (C_{quat.}), 44.9 (CH₂), 32.5 (CH₂), 25.3 (CH₃), 21.0 (CH₃), 2.8 (CH₂) - MS (ESI): m/z (%) = 482.9 (100) [MH⁺], 524.0 (16) [MH⁺+MeCN]

General procedure D for synthesis of Trimethylammoniopropyl-spirobenzopyrans:

A flask was charged with the corresponding 3-iodopropyl-spirobenzopyran (1.0 eq.), capped with rubber septum and flushed with argon. A solution of trimethylamine (66 eq., 33 wt %, in EtOH) was added and the reaction mixture was stirred at room temperature in the dark. Following, the solvent and excess trimethylamine were evaporated under reduced pressure and the crude product was dried in vacuo over night.

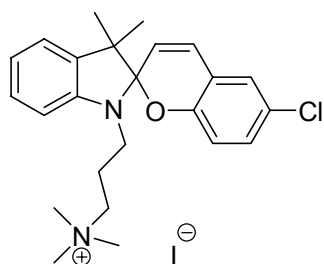
1'-(3''-trimethylammoniopropyl)-3',3'-dimethyl-6-nitro-Spiro[2H-1-benzopyran-2,2'-indoline] iodide



The compound was prepared following general procedure D. Reaction time: 60 hours. Purification by recrystallization from EtOH/Et₂O (twice); pale green crystals; Yield: 79 %.

¹H NMR (300 MHz, DMSO-d₆): δ = 8.25 (d, 1 H, J = 2.8 Hz), 8.01 (dd, 1 H, J = 2.8, 9.0 Hz), 7.24 (d, 1 H, J = 10.4 Hz), 7.15 (d, 2 H, J = 6.9 Hz), 6.91 (d, 1 H, J = 9.0 Hz), 6.84 (t, 1 H, J = 7.3 Hz), 6.73 (d, 1 H, J = 7.9 Hz), 6.09 (d, 1 H, J = 10.4 Hz), 3.27-3.14 (m, 4 H), 2.14-1.86 (m, 2 H), 1.21 (s, 3 H), 1.14 (s, 3 H) - MS (ESI): m/z (%): 408.0 (100) [M⁺]

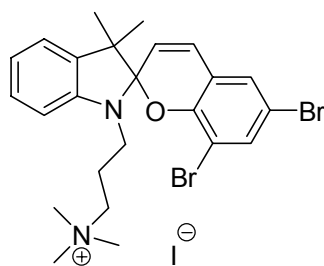
1'-(3''-trimethylammoniopropyl)-3',3'-dimethyl-6-chloro-Spiro[2H-1-benzopyran-2,2'-indoline] iodide



The compound was prepared following general procedure D. Reaction time: 64 hours. For purification CH₂Cl₂ was added and hexane was carefully layered on top to obtain the product; lustrous orange crystals; Yield: 91 %.

^1H NMR (300 MHz, MeOD): δ = 7.18-7.03 (m, 4 H), 6.97 (d, 1 H, J = 10.3 Hz), 6.82 (t, 1 H, J = 7.4 Hz), 6.67 (dd, 2 H, J = 8.2, 16.0 Hz), 5.94 (d, 1 H, J = 10.3 Hz), 3.44-3.28 (m, 4 H), 2.26-2.10 (m, 1 H), 2.09-1.93 (m, 1 H), 1.27 (s, 3 H), 1.17 (s, 3 H) - ^{13}C NMR (75 MHz, MeOD): δ = 154.0 ($\text{C}_{\text{quat.}}$), 148.3 ($\text{C}_{\text{quat.}}$), 137.8 ($\text{C}_{\text{quat.}}$), 130.6 (+, CH), 130.2 (+, CH), 128.8 (+, CH), 127.7 (+, CH), 126.2 ($\text{C}_{\text{quat.}}$), 122.9 (+, CH), 122.1 (+, CH), 121.6 ($\text{C}_{\text{quat.}}$), 120.8 (+, CH), 117.4 (+, CH), 107.9 (+, CH), 106.4 ($\text{C}_{\text{quat.}}$), 66.1 (-, CH_2), 53.8 ($\text{C}_{\text{quat.}}$), 53.4 (+, CH_3), 41.6 (-, CH_2), 26.5 (+, CH_3), 24.1 (-, CH_2), 20.5 (+, CH_3) - MS (ESI): m/z (%): 397.1 (100) [M^+]

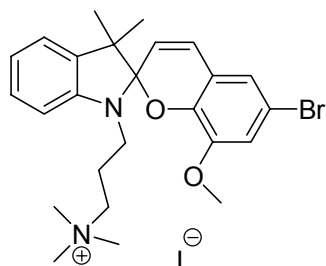
1'-(3''-trimethylammoniopropyl)-3',3'-dimethyl-6,8-dibromo-Spiro[2H-1-benzopyran-2,2'-indoline] iodide



The compound was prepared following general procedure D. Reaction time: 64 hours. For purification a saturated solution of product in MeOH was placed in the freezer and the pure product was obtained; ivory crystals; Yield: 88 %.

^1H NMR (300 MHz, MeOD): δ = 7.50 (d, 1 H, J = 2.3 Hz), 7.32 (d, 1 H, J = 2.3 Hz), 7.15 (dt, 1 H, J = 1.2, 7.7 Hz), 7.09 (d, 1 H, J = 7.3 Hz), 6.96 (d, 1 H, J = 10.3 Hz), 6.83 (t, 1 H, J = 7.4 Hz), 6.72 (d, 1 H, J = 7.8 Hz), 5.96 (d, 1 H, J = 10.3 Hz), 3.48-3.31 (m, 4 H), 2.29-2.12 (m, 1 H), 2.11-1.93 (m, 1 H), 1.29 (s, 3 H), 1.17 (s, 3 H) - ^{13}C NMR (75 MHz, MeOD): δ = 151.0 ($\text{C}_{\text{quat.}}$), 147.8 ($\text{C}_{\text{quat.}}$), 137.5 ($\text{C}_{\text{quat.}}$), 135.9 ($\text{C}_{\text{quat.}}$), 130.1 (+, CH), 129.6 (+, CH), 129.5 (+, CH), 128.9 (+, CH), 123.5 (+, CH), 123.3 ($\text{C}_{\text{quat.}}$), 122.9 (+, CH), 121.0 (+, CH), 113.3 ($\text{C}_{\text{quat.}}$), 110.7 ($\text{C}_{\text{quat.}}$), 107.9 (+, CH), 66.0 (-, CH_2), 53.8 (+, CH_3), 53.6 ($\text{C}_{\text{quat.}}$), 41.5 (-, CH_2), 26.3 (+, CH_3), 23.8 (-, CH_2), 20.7 (+, CH_3) - MS (ESI): m/z (%): 521.1 (100) [M^+]

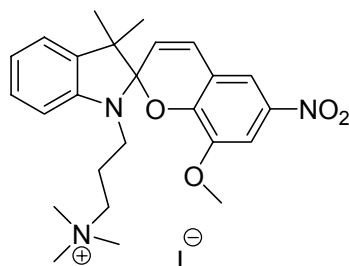
1'-(3''-trimethylammoniopropyl)-3',3'-dimethyl-6-bromo-8-methoxy-Spiro[2H-1-benzopyran-2,2'-indoline] iodide



The compound was prepared following general procedure D. Reaction time: 64 hours. For purification CH_2Cl_2 was added and Et_2O was carefully layered on top to obtain the product; ivory crystals; Yield: 85 %.

^1H NMR (300 MHz, MeOD): δ = 7.13 (dt, 1 H, J = 1.2, 7.7 Hz), 7.06 (dd, 1 H, J = 0.9, 7.3 Hz), 6.95 (dd, 2 H, J = 2.2, 12.0 Hz), 6.90 (d, 1 H, J = 10.3 Hz), 6.81 (t, 1 H, J = 7.4 Hz), 6.66 (d, 1 H, J = 7.8 Hz), 5.86 (d, 1 H, J = 10.2 Hz), 3.70 (s, 3 H), 3.51.3.32 (m, 4 H), 2.26-1.92 (m, 2 H), 1.27 (s, 3 H), 1.14 (s, 3 H) - ^{13}C NMR (75 MHz, MeOD): δ = 149.2 ($\text{C}_{\text{quat.}}$), 148.1 ($\text{C}_{\text{quat.}}$), 143.5 ($\text{C}_{\text{quat.}}$), 137.6 ($\text{C}_{\text{quat.}}$), 129.8 (+, CH), 128.8 (+, CH), 123.0 (+, CH), 122.9 (+, CH), 122.5 (+, CH), 122.3 ($\text{C}_{\text{quat.}}$), 120.6 (+, CH), 117.3 (+, CH), 112.9 ($\text{C}_{\text{quat.}}$), 107.5 (+, CH), 106.3 ($\text{C}_{\text{quat.}}$), 66.1 (-, CH_2), 56.9 (+, CH_3), 53.6 (+, CH_3), 53.5 ($\text{C}_{\text{quat.}}$), 41.2 (-, CH_2), 26.3 (+, CH_3), 24.1 (-, CH_2), 20.5 (+, CH_3) - MS (ESI): m/z (%): 473.1 (100) [M^+]

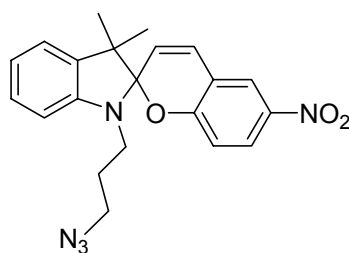
1'-(3''-trimethylammoniopropyl)-3',3'-dimethyl-6-nitro-8-methoxy-Spiro[2H-1-benzopyran-2,2'-indoline] iodide



The compound was prepared following general procedure D. Reaction time: 64 hours. For purification CH_2Cl_2 was added and Et_2O was carefully layered on top to grow crystals. Following, trituration with Et_2O ; green crystals; Yield: 89 %.

^1H NMR (300 MHz, DMSO-d_6): δ = 7.93 (d, 1 H, J = 2.6 Hz), 7.67 (d, 1 H, J = 2.6 Hz), 7.23-7.11 (m, 3 H), 6.83 (t, 1 H, J = 7.4 Hz), 6.74 (d, 1 H, J = 7.7 Hz), 6.08 (d, 1 H, J = 10.4 Hz), 3.78 (s, 3 H), 3.40-3.31 (m, 4 H), 2.13-1.88 (m, 2 H), 1.19 (s, 3 H), 1.13 (s, 3 H) - ^{13}C NMR (75 MHz, DMSO-d_6): δ = 148.4 ($\text{C}_{\text{quat.}}$), 146.6 ($\text{C}_{\text{quat.}}$), 146.4 ($\text{C}_{\text{quat.}}$), 139.9 ($\text{C}_{\text{quat.}}$), 135.6 ($\text{C}_{\text{quat.}}$), 128.2 (+, CH), 127.5 (+, CH), 121.8 (+, CH), 121.6 (+, CH), 119.3 (+, CH), 118.3 ($\text{C}_{\text{quat.}}$), 115.3 (+, CH), 107.3 (+, CH), 106.7 (+, CH), 106.3 ($\text{C}_{\text{quat.}}$), 63.3 (-, CH_2), 55.9 (+, CH_3), 52.2 (+, CH_3), 52.1 ($\text{C}_{\text{quat.}}$), 40.1 (-, CH_2), 25.8 (+, CH_3), 22.1 (-, CH_2), 19.4 (+, CH_3) - MS (ESI): m/z (%): 438.2 (100) [M^+]

1'-(3-Azidopropyl)-1',3'-dihydro-3',3'-dimethyl-6-nitro-Spiro[2H-1-benzopyran-2,2'[2H]indole]

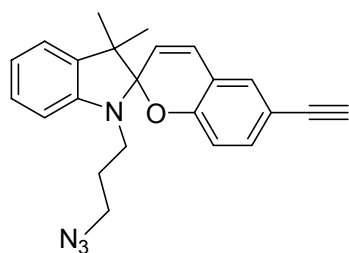


1'-(3-Iodopropyl)-1',3'-dihydro-3',3'-dimethyl-6-nitro-Spiro[2H-1-benzopyran-2,2'[2H]indole] (1.576 g, 3.31 mmol) was dissolved in dry N,N -Dimethylformamide (62 mL). Following,

NaN_3 (877 mg, 13.49 mmol) was added in one portion and the reaction mixture was stirred in the dark at room temperature for 19 hours. The solvent was evaporated under reduced pressure, the residue was dried in vacuo and the crude product was purified by flash chromatography on silica gel (CH_2Cl_2) to yield 1'-(3-Azidopropyl)-1',3'-dihydro-3',3'-dimethyl-6-nitro-Spiro[2H-1-benzopyran-2,2'[2H]indole] as glistening golden foam (1.076 g, 83 %). $R_f = 0.82$ (CH_2Cl_2)

^1H NMR (400 MHz, CDCl_3): $\delta = 8.01$ (m, 2 H, H-Ar), 7.20 (dt, 1 H, $J = 1.3, 7.7$ Hz, H-Ar), 7.10 (dd, 1 H, $J = 0.9, 7.3$ Hz, H-Ar), 6.94 (d, 1 H, $J = 10.3$ Hz, H-Ar), 6.90 (dt, 1 H, $J = 0.9, 7.5$ Hz, H-Ar), 6.75 (d, 1 H, $J = 8.4$ Hz, H-Ar), 6.60 (d, 1 H, $J = 7.8$ Hz, H-Ar), 5.86 (d, 1 H, $J = 10.4$ Hz, H-Ar), 3.39-3.18 (m, 4 H, 2 x CH_2), 2.01-1.90 (m, 1 H, CH_2 -propyl), 1.88-1.75 (m, 1 H, CH_2 -propyl), 1.29 (s, 3 H, CH_3), 1.19 (s, 3 H, CH_3) - ^{13}C NMR (100 MHz, CDCl_3): $\delta = 159.4$ ($\text{C}_{\text{quat.}}$), 146.8 ($\text{C}_{\text{quat.}}$), 141.1 ($\text{C}_{\text{quat.}}$), 136.0 ($\text{C}_{\text{quat.}}$), 128.4 (CH), 127.8 (CH), 125.9 (CH), 122.8 (CH), 121.8 (CH), 121.6 (CH), 119.8 (CH), 118.4 ($\text{C}_{\text{quat.}}$), 115.5 (CH), 106.7 (CH), 106.6 ($\text{C}_{\text{quat.}}$), 52.6 ($\text{C}_{\text{quat.}}$), 49.0 (CH_2), 40.8 (CH_2), 28.1 (CH_2), 25.9 (CH_3), 19.9 (CH_3) - IR (neat): ν [cm^{-1}] = 2970, 2359, 2097, 1738, 1477, 1333 - MS (ESI): m/z (%): 392.0 (100) [MH^+] - HRMS (PI-EI) calcd. for $\text{C}_{21}\text{H}_{21}\text{N}_5\text{O}_3$ [M^+]: 391.1644, found: 391.1644

1'-(3-Azidopropyl)-1',3'-dihydro-3',3'-dimethyl-6-ethynyl-Spiro[2H-1-benzopyran-2,2'-[2H]indole]

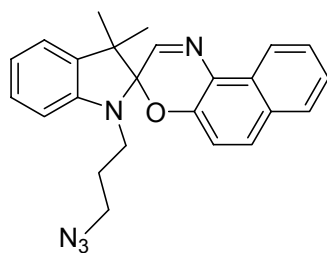


1'-(3-Iodopropyl)-1',3'-dihydro-3',3'-dimethyl-6-ethynyl-Spiro[2H-1-benzopyran-2,2'-[2H]indole] (264 mg, 0.58 mmol) was dissolved in dry N,N -Dimethylformamide (13 mL). Following, NaN_3 (204 mg, 3.14 mmol) was added in one portion and the reaction mixture was stirred in the dark at room temperature for 48 hours. The solvent was evaporated under reduced pressure, the residue was dissolved in a mixture of CH_2Cl_2 and water. The aqueous layer was extracted twice with CH_2Cl_2 , the organic layers were combined and dried over

anhydrous Na_2SO_4 . The product was purified by gradient flash chromatography on silica gel (CH_2Cl_2 to $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 50:1) to yield 1'-(3-Azidopropyl)-1',3'-dihydro-3',3'-dimethyl-6-ethynyl-Spiro[2H-1-benzopyran-2,2'-[2H]indole] as a pale yellow-green fluffy foam (207 mg, 96 %). $R_f = 0.86$ (CH_2Cl_2)

^1H NMR (300 MHz, CDCl_3): $\delta = 7.30$ -7.19 (m, 4 H), 6.87 (dd, 2 H, $J = 8.7, 15.2$ Hz), 6.62 (dd, 2 H, $J = 7.9, 18.0$ Hz), 5.73 (d, 1 H, $J = 10.3$ Hz), 3.49-3.33 (m, 4 H), 3.00 (s, 1 H), 1.98-1.92 (m, 1 H), 1.87-1.79 (m, 1 H), 1.31 (s, 3 H), 1.19 (s, 3 H) - MS (ESI): m/z (%): 371.1 (100) $[\text{MH}^+]$

1'-(3-Azidopropyl)-1',3'-dihydro-3'.3'-dimethyl-spiro[3H]naphth[2,1-b][1,4]oxazine



1'-(3-Iodopropyl)-1',3'-dihydro-3'.3'-dimethyl-spiro[3H]naphth[2,1-b][1,4]oxazine (119 mg, 0.25 mmol) was dissolved in dry N,N-Dimethylformamide (3.0 mL). Following, NaN_3 (64 mg, 0.985 mmol) was added in one portion and the reaction mixture was stirred in the dark at room temperature for 48 hours. The solvent was evaporated under reduced pressure, the residue was dissolved in a mixture of CH_2Cl_2 (25 mL) and water (30 mL). The aqueous layer was extracted again with CH_2Cl_2 (25 mL), the organic layers were pooled and dried over anhydrous Na_2SO_4 . The product was purified by gradient flash chromatography on silica gel (CH_2Cl_2 to $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 1000:1) to yield 1'-(3-Azidopropyl)-1',3'-dihydro-3'.3'-dimethyl-spiro[3H]naphth[2,1-b][1,4]oxazine as a pale yellow powder (82 mg, 84 %). $R_f = 0.83$ (CH_2Cl_2)

^1H NMR (600 MHz, CDCl_3): $\delta = 8.56$ (d, 1 H, $J = 8.4$ Hz, H-Ar), 7.75 (d, 1 H, $J = 8.1$ Hz, H-Ar), 7.68 (d, 1 H, $J = 8.9$ Hz, H-Ar), 7.58 (ddd, 1 H, $J = 1.1, 6.9, 8.2$ Hz, H-Ar), 7.41 (ddd, 1 H, $J = 1.1, 7.0, 8.0$ Hz, H-Ar), 7.22 (dt, 1 H, $J = 1.1, 7.7$ Hz, H-Ar), 7.09 (dd, 1 H, $J = 0.8, 7.2$ Hz, H-Ar), 7.00 (d, 1 H, $J = 8.8$ Hz, H-Ar), 6.91 (t, 1 H, $J = 7.4$ Hz, H-Ar), 6.63 (d, 1 H, $J =$

7.8 Hz, H-Ar), 3.39-3.22 (m, 4 H, 2 x CH₂-propyl), 2.01-1.95 (m, 1 H, CH₂-propyl), 1.89-1.83 (m, 1 H, CH₂-propyl), 1.36 (s, 3 H, CH₃), 1.34 (s, 3 H, CH₃) - ¹³C NMR (150 MHz, CDCl₃): δ = 150.9 (CH), 146.7 (C_{quat.}), 143.8 (C_{quat.}), 135.6 (C_{quat.}), 130.8 (C_{quat.}), 130.4 (CH), 129.3 (C_{quat.}), 128.0 (CH), 127.8 (CH), 127.2 (CH), 124.2 (CH), 121.8 (CH), 121.5 (CH), 119.8 (CH), 116.8 (CH), 106.8 (CH), 99.0 (C_{quat.}), 52.1 (C_{quat.}), 49.1 (CH₂), 41.5 (CH₂), 29.7 (C_{quat.}), 28.2 (CH₂), 25.3 (CH₃), 21.0 (CH₃) - IR (neat): ν [cm⁻¹] = 2959, 2926, 2854, 2095, 1483 - MS (ESI): m/z (%): 398.0 (100) [MH⁺], 439.1 (42) [MH⁺+MeCN]

1.5. References

- [1] H. Bouas-Laurent, H. Dürr, *Pure Appl. Chem.* **2001**, 73, 639.
- [2] M. Maafi, *Molecules* **2008**, 13, 2260.
- [3] M. Tomasulo, F. M. Raymo, *J. Phys. Chem. A* **2005**, 109, 7343.
- [4] H. Morrison, *Biological applications of photochemical switches* **1993**, John Wiley & Sons.
- [5] a)R. R. Rando, *Angew. Chem. Int. Ed. Engl.* **1990**, 29, 461; b)F. M. Raymo, S. Giordani, *Org. Lett.* **2001**, 3, 3475; c)F. M. Raymo, *The Spectrum* **2004**, 14.
- [6] H. Nakanishi, *Prog. Surf. Sci.* **1995**, 49, 197.
- [7] a)H. Nakamichi, T. Okada, *Angew. Chem. Int. Ed. Engl.* **2006**, 45, 4270; b)O. Weingart, *J. Am. Chem. Soc.* **2007**, 129, 10618.
- [8] a)S. L. Gilat, S. H. Kawai, J.-M. Lehn, *Chem. Eur. J.* **1995**, 1, 275; b)Y. Yokoyama, Y. Kurita, *Mol. Cryst. Liq. Cryst.* **1994**, 246, 87.
- [9] a)P. Gorostiza, E. Y. Isacoff, *Science* **2008**, 322, 395; b)S. Yasuda, T. Nakamura, M. Matsumoto, H. Shigekawa, *J. Am. Chem. Soc.* **2003**, 125, 16430.
- [10] a)T. Kawato, H. Kanatomi, H. Koyama, T. Igarashi, *J. Photochem. Photobiol., A* **1986**, 33, 199; b)X.-C. Tang, D.-Z. Jia, K. Liang, X.-G. Zhang, X. Xia, Z.-Y. Zhou, *J. Photochem. Photobiol., A* **2000**, 134, 23.
- [11] H. Bouas-Laurent, A. Castellan, J. P. Desvergne, R. Lapouyade, *Chem. Soc. Rev.* **2000**, 29, 43.
- [12] J. Andreasson, S. D. Straight, G. Kodis, C.-D. Park, M. Hambourger, M. Gervaldo, B. Albinsson, T. Moore, A. L. Moore, D. Gust, *J. Am. Chem. Soc.* **2006**, 128, 16259.
- [13] S. Nakamura, M. Irie, *J. Org. Chem.* **1988**, 53, 6136.

- [14] a)X. Liang, N. Takenaka, H. Nishioka, H. Asanuma, *Chem. Asian J.* **2008**, *3*, 553; b)H. Asanuma, T. Yoshida, X. Liang, M. Komiyama, *Chem. Lett.* **2000**, 108; c)H. Asanuma, X. Liang, H. Nishioka, D. Matsunaga, M. Liu, M. Komiyama, *Nat. Protoc.* **2007**, *2*, 203.
- [15] a)Y. Saito, K. Matsumoto, Y. Takeuchi, S. S. Bag, S. Kodate, T. Morii, I. Saito, *Tetrahedron Lett.* **2009**, *50*, 1403; b)S. Ogasawara, M. Maeda, *Angew. Chem., Int. Ed.* **2008**, *47*, 8839.
- [16] a)E. J. Corey, *J. Org. Chem.* **2004**, *69*, 2917; b)R. Hoffmann, *Angew. Chem. Int. Ed. Engl.* **2004**, *43*, 6586.
- [17] N. Tamai, H. Miyasaka, *Chem. Rev.* **2000**, *100*, 1875.
- [18] M. Irie, M. Mohri, *J. Org. Chem.* **1988**, *53*, 803.
- [19] J. Daub, T. Knöchel, A. Mannschreck, *Angew. Chem. Int. Ed. Engl.* **1984**, *23*, 960.
- [20] G. Berkovic, V. Krongauz, V. Weiss, *Chem. Rev.* **2000**, *100*, 1741.
- [21] Y. Yokoyama, *Chem. Rev.* **2000**, *100*, 1717.
- [22] a)H. G. Heller, R. M. Megit, *J. Chem. Soc., Perkin Trans I* **1974**, 923; b)H. G. Heller, S. Oliver, *J. Chem. Soc., Perkin Trans I* **1981**, 197.
- [23] A. P. Glaze, S. A. Harris, H. G. Heller, W. Johncock, S. N. Oliver, P. J. Strydom, J. Whittall, *J. Chem. Soc., Perkin Trans I* **1985**, 957.
- [24] M. Irie, *Chem. Rev.* **2000**, *100*, 1685.
- [25] V. de Waele, U. Schmidhammer, T. Mrozek, J. Daub, E. Riedle, *J. Am. Chem. Soc.* **2002**, *124*, 2438.
- [26] a)M. Sørensen, H. Autrup, P. Møller, O. Hertel, S. S. Jensen, P. Vinzents, L. E. Knudsen, S. Lof, *Mutation Research* **2003**, *544*, 255; b)K. B. Okona-Mensah, J. Battershill, A. Boobis, R. Fielder, *Food Chem. Toxicol.* **2005**, *43*, 1103.
- [27] F. Xie, K. Sivakumar, Q. Zeng, M. A. Bruckman, B. Hodges, Q. Wang, *Tetrahedron* **2008**, *64*, 2906.
- [28] H. D. Becker, *Chem. Rev.* **1993**, *93*, 145.
- [29] a)J. H. R. Tucker, H. Bouas-Laurent, P. Marsau, S. W. Riley, J.-P. Desvergne, *Chem. Commun.* **1997**, 1165; b)G. Deng, T. Sakaki, Y. Kawahara, S. Shinkai, *Tetrahedron Lett.* **1992**, *33*, 2163.
- [30] D. S. Tyson, C. A. Bignozzi, F. N. Castellano, *J. Am. Chem. Soc.* **2002**, *124*, 4562.
- [31] H. Dürr, H. Bouas-Laurent, *Photochromism: molecules and systems, 1st ed.* **2003**, Elsevier.

- [32] a)U. Pischel, *Angew. Chem., Int. Ed.* **2007**, *46*, 4026; b)K. Szacilowski, *Chem. Rev.* **2008**, *108*, 3481.
- [33] a)A. Koçer, M. Walko, W. Meijberg, B. L. Feringa, *Science* **2005**, 755; b)I. Vlassiouk, C.-D. Park, S. A. Vail, D. Gust, S. Smirnov, *Nano Lett.* **2006**, *6*, 1013.
- [34] D. D. Young, A. Deiters, *ChemBioChem* **2008**, *9*, 1225.
- [35] a)Y. Hirshberg., E. Fischer, *J. Chem. Soc.* **1952**, 4522; b)Y. Hirshberg., *J. Am. Chem. Soc.* **1956**, *78*, 2304.
- [36] a)D. A. Parthenopoulos, P. M. Rentzepis, *Science* **1989**, *245*, 843; b)S. Kawata, Y. Kawata, *Chem. Rev.* **2000**, *100*, 1777.
- [37] J. Hobley, U. Pfeifer-Fukumura, M. Bletz, T. Asahi, H. Masuhara, H. Fukumura, *J. Phys. Chem. A* **2002**, *106*, 2265.
- [38] B. L. Feringa, W. F. Jager, B. de Lange, *Tetrahedron* **1993**, *49*, 8267.
- [39] B. L. Feringa, R. A. van Delden, N. Koumura, E. M. Geertsema, *Chem. Rev.* **2000**, *100*, 1789.
- [40] B. Stephan, A. Mannschreck, N. A. Voloshin, N. V. Volbushko, V. I. Minkin, *Tetrahedron Lett.* **1990**, *31*, 6335.
- [41] X. Meng, W. Zhu, Z. Guo, J. Wang, H. Tian, *Tetrahedron* **2006**, *62*, 9840.
- [42] J. C. Crano, R. J. Guglielmetti, *Organic Photochromic and Thermochromic Compounds, Vol. 1* **1999**, Springer, Berlin.
- [43] a)V. F. Traven, V. S. Miroshnikov, T. A. Chibisova, V. A. Barachevsky, O. V. Venidiktova, Y. P. Stokach, *Russ. Chem. Bull., Int. Ed.* **2005**, *54*, 2417; b)R. Muthyala, *Chemistry and applications of leuco dyes, 1st ed.* **1997**, Plenum Press.
- [44] M. V. Kvach, A. V. Ustinov, I. A. Stepanova, A. D. Malakhov, M. V. Skorobogaty, V. V. Shmanai, V. A. Korshun, *Eur. J. Org. Chem.* **2008**, 2107.
- [45] a)S. R. Torres, A. L. Vazquez, E. A. Gonzalez, *Synth. Commun.* **1995**, *25*, 105; b)E. A. Gonzalez, M. J. G. Lozano, *Synth. Commun.* **1998**, *28*, 4035.
- [46] a)S. Kumar, D. L. Watkins, T. Fujiwara, *Chem. Commun.* **2009**, 4369; b)H. Durr, Y. Ma, G. Cortellaro, *Synthesis* **1995**, 294.
- [47] a)T. Deligeorgiev, S. Minkovska, B. Jejiakova, S. Rakovsky, *Dyes Pigm.* **2002**, *53*, 101; b)N. Y. C. Chu, *Can. J. Chem.* **1983**, *61*, 300.
- [48] A. V. Koshkin, O. A. Fedorova, V. Lokshin, R. Guglielmetti, J. Hamelin, F. Texier-Boullet, S. P. Gromo, *Synth. Commun.* **2004**, *34*, 315.
- [49] Y. Lin, R. Weissleder, C.-H. Tung, *Bioconjugate Chem.* **2002**, *13*, 605.
- [50] P. Zhang, J. Meng, X. Li, T. Matsuura, Y. Wang, *J. Heterocycl. Chem.* **2002**, *39*, 179.

- [51] F. M. Raymo, S. Giordani, *J. Am. Chem. Soc.* **2001**, *123*, 4651.
- [52] G. Cravotto, A. Demetri, G. M. Nano, G. Palmisano, A. Penoni, S. Tagliapietra, *Eur. J. Org. Chem.* **2003**, 4438.
- [53] a) J. Leonard, B. Lygo, G. Procter, *Advanced practical organic chemistry* **1998**, Taylor & Francis CRC Press; b) D. Bierne, S. Smith, B. E. Hoogenboom, *J. Chem. Educ.* **1974**, *51*, 602; c) R. E. R. Craig, *J. Chem. Educ.* **1989**, *66*, 88.
- [54] a) W. C. Still, M. Kahn, A. Mitra, *J. Org. Chem.* **1978**, *43*, 2923; b) B. A. Jacobson, *J. Chem. Educ.* **1988**, *65*, 459.
- [55] L. M. Harwood, *Aldrichimica Acta* **1985**, *18*, 25.
- [56] K.-H. Chang, C.-C. Huang, Y.-H. Liu, Y.-H. Hu, P.-T. Chou, Y.-C. Lin, *Dalton Trans.* **2004**, 1731.
- [57] a) J. E. Hein, V. V. Fokin, *Chem. Soc. Rev.* **2010**, 1302; b) R. M. El-Shishtawy, *Int. J. Photoenergy* **2009**, 1.
- [58] A. K. Chibisov, H. Görner, *Chem. Phys.* **1998**, *237*, 425.
- [59] C. Reichardt, *Chem. Rev.* **1994**, *94*, 2319.
- [60] J. W. Verhoeven, *Pure Appl. Chem.* **1996**, *68*, 2223.
- [61] S. Wang, S.-H. Kim, *Dyes Pigm.* **2009**, *80*, 314.
- [62] a) K.-C. Feng, J. Griffiths, *Adv. Col. Sci. Technol.* **2001**, *4*, 12; b) H. Mustroph, J. Mistol, B. Senns, D. Keil, M. Findeisen, L. Hennig, *Angew. Chem. Int. Ed. Engl.* **2009**, *48*, 8773.
- [63] a) J. Andreasson, J. Andersson, P. Lincoln, S. Li, *Nucleic Acids Symp. Ser.* **2008**, 675; b) J. Andersson, S. Li, P. Lincoln, J. Andreasson, *J. Am. Chem. Soc.* **2008**, *130*, 11836.
- [64] P. M. E. Gramlich, S. Warncke, J. Gierlich, T. Carell, *Angew. Chem., Int. Ed.* **2008**, *47*, 3442.
- [65] a) W. G. Lewis, L. G. Green, F. Grynszpan, Z. Radicacute, P. R. Carlier, P. Taylor, M. G. Finn, K. B. Sharpless, *Angew. Chem. Int. Ed. Engl.* **2002**, *41*, 1053; b) C. W. Tornøe, C. Christensen, M. Meldal, *J. Org. Chem.* **2002**, *67*, 3057; c) P. Ball, *Chemistry World* **2007**, *4*, 46.
- [66] M. Noskowska, E. Sliwinska, W. Duczmal, *Transition Met. Chem.* **2003**, *28*, 756.
- [67] a) D. D. Perrin, W. L. F. Armarego, D. R. Perrin, *Purification of Laboratory Chemicals*, 2nd ed. **1987**, Pergamon, Frankfurt; b) S. Hünig, P. Kreitmeier, G. Märkl, J. Sauer, *Arbeitsmethoden in der organischen Chemie*, 1st ed. **2006**, Lehmanns, Berlin.

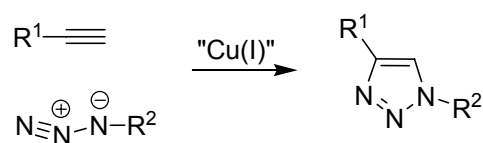
- [68] a) A. J. Shusterman, P. G. McDougal, A. Glasfeld, *J. Chem. Educ.* **1997**, *74*, 1222;
b) D. S. Pedersen, C. Rosenbohm, *Synthesis* **2001**, *16*, 2431.

Chapter 2:

Synthesis of a spirobenzopyran azide and alkyne fluorescent dyes - “click”-type synthesis and optical properties of molecular dyads

2.1. Introduction

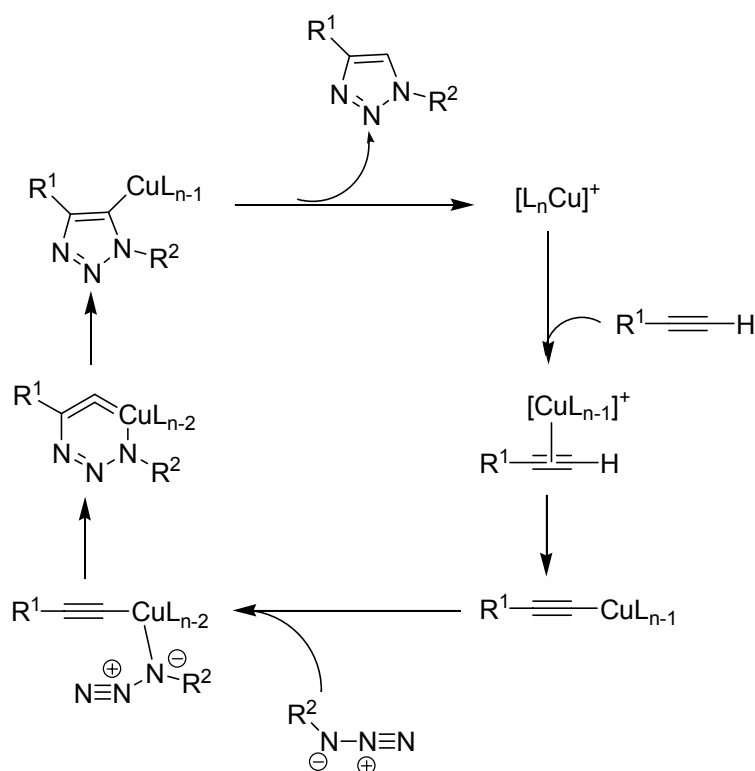
In 2002, Meldal and Sharpless independently reported the regioselective formation of 1,4-disubstituted 1,2,3-triazoles in a Huisgen 1,3-dipolar cycloaddition of terminal acetylenes with azides under copper(I)-catalysis.^[1] Although other chemical reactions have been evaluated and discussed as key steps for fast and modular approaches, none of them were able to beset the indefeasible stardom of this reaction.^[2] Since the discovery of this copper(I)-catalyzed azide/alkyne cycloaddition (CuAAC), the reaction has become very popular under the catchphrase “click” reaction, and the interest and applications have increased dramatically, with no sign of slowing down.^[3]



Scheme 1.1. Copper(I)-catalyzed synthesis of 1,4-substituted 1,2,3-triazole^[4]

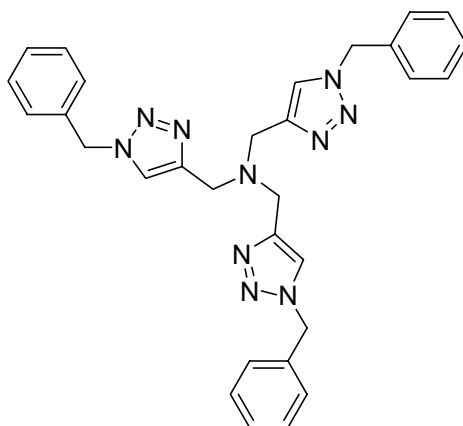
Experimentally, the click process itself is very forgiving and only requires benign reaction and workup conditions. However, it still can rapidly create molecular diversity through the use of its molecular building blocks and therefore appears to have enormous scope.

The mechanism leading to the 1,4-regioselectivity for 1,2,3-triazole formation begins with addition of copper(I) to the terminal acetylene resulting in the formation of the copper(I) acetylide through abstraction of a proton and displacement of one ligand. For the next step it is proposed that the azide replaces another copper ligand and binds to the copper with the nitrogen proximal to the carbon atom. Following, the distal nitrogen of the azide attacks on the C-2 carbon of the acetylide, proceeding via a six-membered copper(III) intermediate. After that, ring contraction forms the triazolyl-copper intermediate from which the desired triazole product is released by proteolysis.^[1a, 5] Despite other proposed outlines for the dynamically changing family of different copper(I) species and mechanisms,^[6] the CuAAC entails the 1,4-regioselective formation of 1,2,3-triazole via a stepwise mechanism.



Scheme 1.2. Outline of a proposed catalytic cycle for CuAAC reaction^[5]

Since no systematic study of optimal conditions have been reported, particularly with respect to the formation of the active copper(I) species, conditions have varied widely.^[7] Excellent copper(I) sources have been found by in situ reduction of copper(II) sulphate or copper(II) acetate using the reductant ascorbic acid or sodium ascorbate. Among the myriad of CuAAC reactions, the combination of sodium ascorbate and copper(II) sulphate hydrate is the most utilized way to produce the active copper(I) species.^[8] Other sources include copper(I) salts, most commonly copper iodide. In addition, most click reactions also demand the use of bases such as triethylamine, DIPEA or 2,6-lutidine.^[7] Due to the general thermodynamic instability of copper(I), other additives and ligands bearing triazole moieties themselves have been shown to be capable of stabilizing the copper(I) species and therefore also increase reaction rates; e.g. the most commonly used polytriazole additive TBTA features three triazole moieties and a central tertiary nitrogen atom.



Scheme 1.3. TBTA, a copper(I) stabilizing ligand

TBTA precludes the need for additional base and its tetradentate binding ability is believed to completely shield the copper(I) from interactions that would lead to destabilizing interactions or degradation.^[4] While addition of TBTA can improve certain aspects of the CuAAC reaction such as protecting copper(I) oxidation or disproportionation,^[9] it should be mentioned that use of TBTA also may complicate simple workup and purification steps, major aspects that, in the first place, drew the attention of many chemists towards click chemistry.

The impressive versatility of the click reaction was already utilized in broad fields, such as chemical synthesis,^[10] labeling^[11] and bioconjugation techniques^[12] and material science.^[13] The use of click chemistry and CuAAC with respect towards DNA is discussed in Chapters 3 and 4.

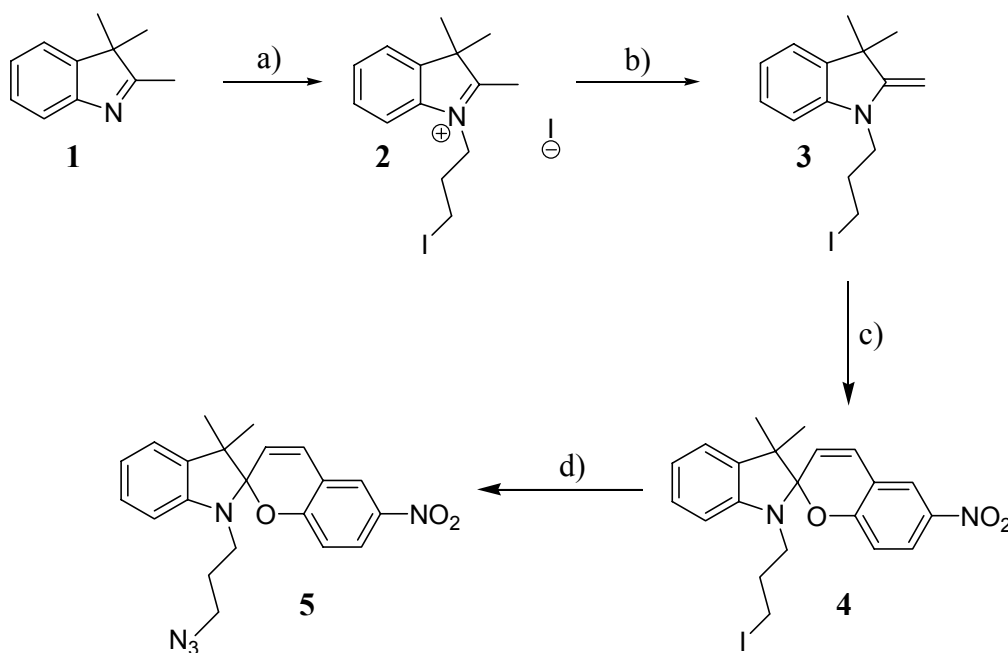
Until now, the spectrum of click chemistry has been further explored and expanded in view of triazole formation, e.g. by use of internal alkynes in the copper-catalyzed transformation^[14] and establishing the regioselective synthesis of 1,5-disubstituted and 1,4,5-trisubstituted 1,2,3-triazoles from azides and terminal and internal alkynes employing Ru-catalysis.^[15]

Herein, we present the Huisgen-Meldal-Sharpless click reaction as a facile methodology providing a new efficient avenue to construct molecular dyads,^[16] bearing a photochromic spirobenzopyran and fluorescent chromophores (pyren, perylene or nile red). Interestingly, the application of CuAAC has rarely been applied for spirobenzopyran conjugation, where only one example has been reported in the literature up to now. There, click chemistry was applied for the formation of supramolecular photochromic dendrimers.^[17] However, until now there has been no precedent on the click synthesis of spirobenzopyran bearing dyads.

2.2. Results and Discussion

2.2.1. Synthesis of a spirobenzopyran azide

In order to synthesize the desired dyads we decided to perform CuAAC with a spirobenzopyran azide and fluorescent chromophores bearing terminal alkyne groups. The spirobenzopyran **5** was chosen as the azide building block for the following click reactions. **5** was prepared in four steps with an overall yield of 55 %, according to the synthetic protocol that is discussed in detail in Chapter 1.

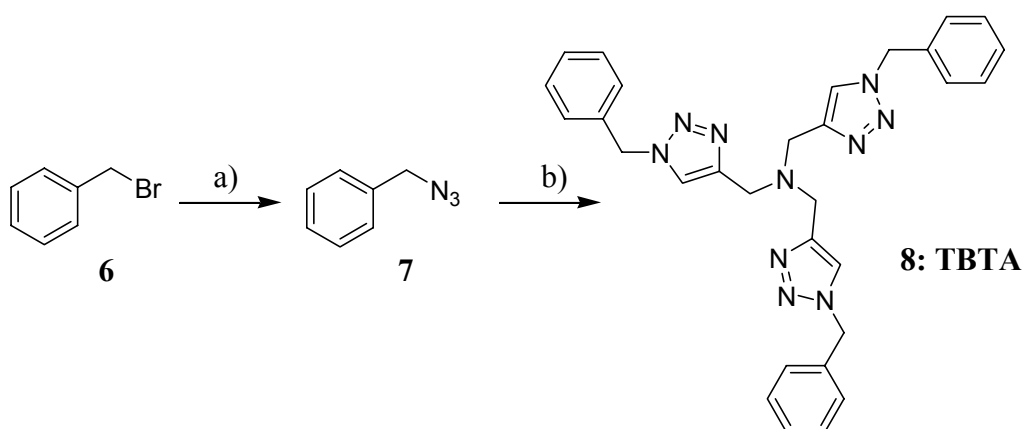


Scheme 2.1. Synthesis of **5**. Reagents and conditions: a) I(CH₂)₂I (3.5 eq.), MeCN, reflux, 48 h, 77 %; b) NaOH (30 eq.), 80 °C to r.t., 1 h, 91 %; c) 2-hydroxy-5-nitro-benzaldehyde (1.0 eq.), EtOH, 1 h, 94 %; d) NaN₃ (4.1 eq.), DMF, r.t., 19 h, 83 %

2.2.2. Synthesis of benzylazide and TBTA

The synthesis of the copper(I) stabilizing ligand TBTA **8** was performed in two steps. A plethora of synthetic procedures for the approach of alkyl azides have been described. Among those, the nucleophilic substitution of bromide with lithium azide or sodium azide from the corresponding alkyl bromide is the most common procedure. Benzyl azide **7** was therefore synthesized by a modified procedure reported in the literature.^[18] A stock solution of sodium azide in DMSO was prepared and subsequently freshly distilled benzyl bromide **6** was added.

After two hours, successful transformation of the bromide to the azide was found and benzyl azide **7** was afforded in 96 % yield.

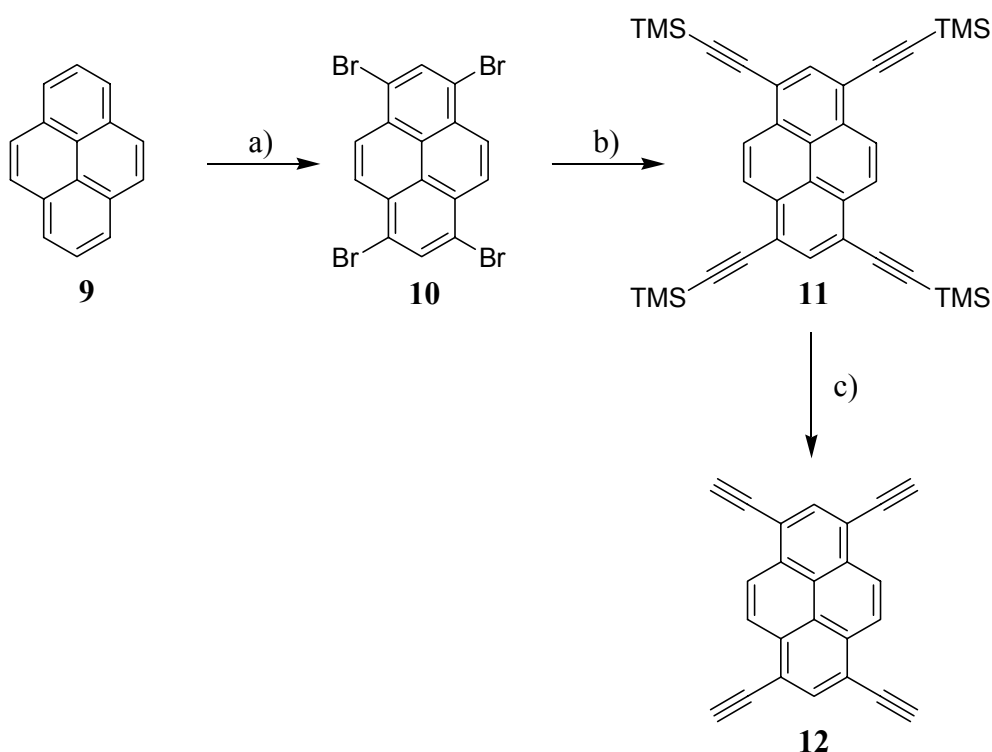


Scheme 2.2. Synthesis of TBTA **8**. Reagents and conditions: a) NaN₃, Bz-Br (1.3 eq.), r.t., 2 h, 96 %; b) Propargylamine (1.0 eq.), Bz-N₃ (3.3 eq.), CuSO₄ (16 mol-%), L-ascorbic acid (44 mol-%), NaOH (44 mol-%), H₂O, CH₂Cl₂, r.t., 14 h, 96 %.

In the second step, TBTA was then synthesized by a modified procedure with use of dichloromethane as a co-solvent with water through the copper(I)-catalyzed 1,3-dipolar cycloaddition of propargylamine and benzyl azide **7**. Although the reaction displayed complete conversion upon TLC control, purification was performed with use of flash chromatography to separate excess benzyl azide. An assimilable excellent yield of 96 % was obtained for TBTA (lit.:98 %)^[19].

2.2.3. Synthesis of 1,3,6,8-Tetra-ethynyl-pyrene

Starting from pyrene **9**, 1,3,6,8-tetrabromopyrene **10** was synthesized by exhaustive bromination using 4.3 equivalents of bromine in nitrobenzene at 140 °C and obtained as a yellow solid (92 %).^[20] In the next step, the Sonogashira cross-coupling reaction was performed with little excess of ethynyltrimethylsilane, while PdCl₂(PPh₃)₂ and copper iodide were used as catalysts. The corresponding tetrakis(trimethylsilylethynyl)pyrene product **11** was achieved as lustrous orange crystals in 78 % yield and showed good solubility in common organic solvents such as dichloromethane, toluene, tetrahydrofuran and chloroform. Consequent desilylation went smoothly by using potassium carbonate in a mixture of methanol and tetrahydrofuran. There, the desired tetraethynylpyrene **12** precipitated and could be obtained in excellent yield after filtration and washing.

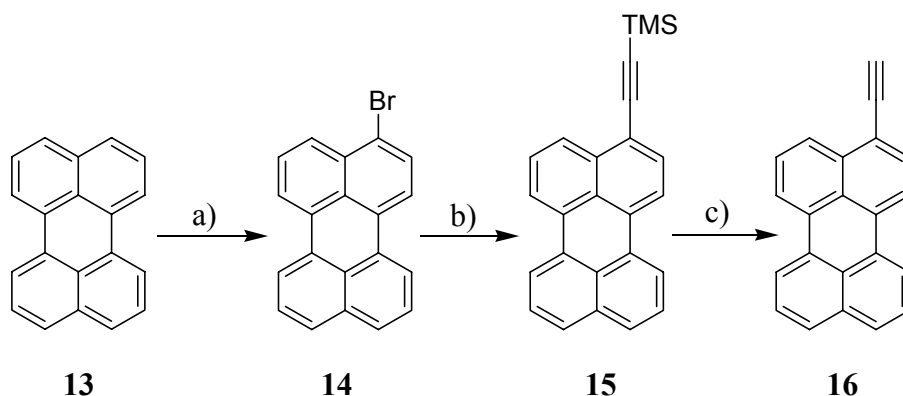


Scheme 2.3. Synthesis of 1,3,6,8-tetraethynylpyrene **12**. Reagents and conditions: a) Br_2 (4.3 eq.), nitrobenzene, 4 h, 140 °C, 92 %; b) $(\text{CH}_3)_3\text{SiCCH}$ (6.1 eq.), $\text{PdCl}_2(\text{PPh}_3)_2$ (20.4 mol-%), CuI (9.9 mol-%), PPh_3 (20.4 mol-%), DMF, NEt_3 , 70 °C, 20 h, 78 %; c) K_2CO_3 (8.0 eq.), MeOH, THF, r.t., 16 h, 95 %.

2.2.4. Synthesis of 3-Ethynylperylene

3-Ethynylperylene **16** was prepared in three steps and the synthetic approach starts from the route published in the literature.^[21] Our approach entails three synthetic steps, starting with commercially available perylene.

First, bromination of perylene **13** with N-bromosuccinimide in DMF gave 3-bromoperylene **14**, according to a procedure described in the literature in 94 % yield.^[22] Deviant from the literature, 3-trimethylsilylethynylperylene **15** was prepared by a Sonogashira cross-coupling reaction in the presence of $\text{PdCl}_2(\text{PPh}_3)_2$ and copper iodide as catalysts, in a heated DMF trimethylamine mixture, and gave **15** in 97 % yield as lustrous yellow-orange crystals. Final cleavage of the TMS protecting group of compound **15** with K_2CO_3 in a methanol tetrahydrofuran mixture was found suitable and afforded 3-ethynylperylene **16** in excellent yield as glistening yellow crystals.

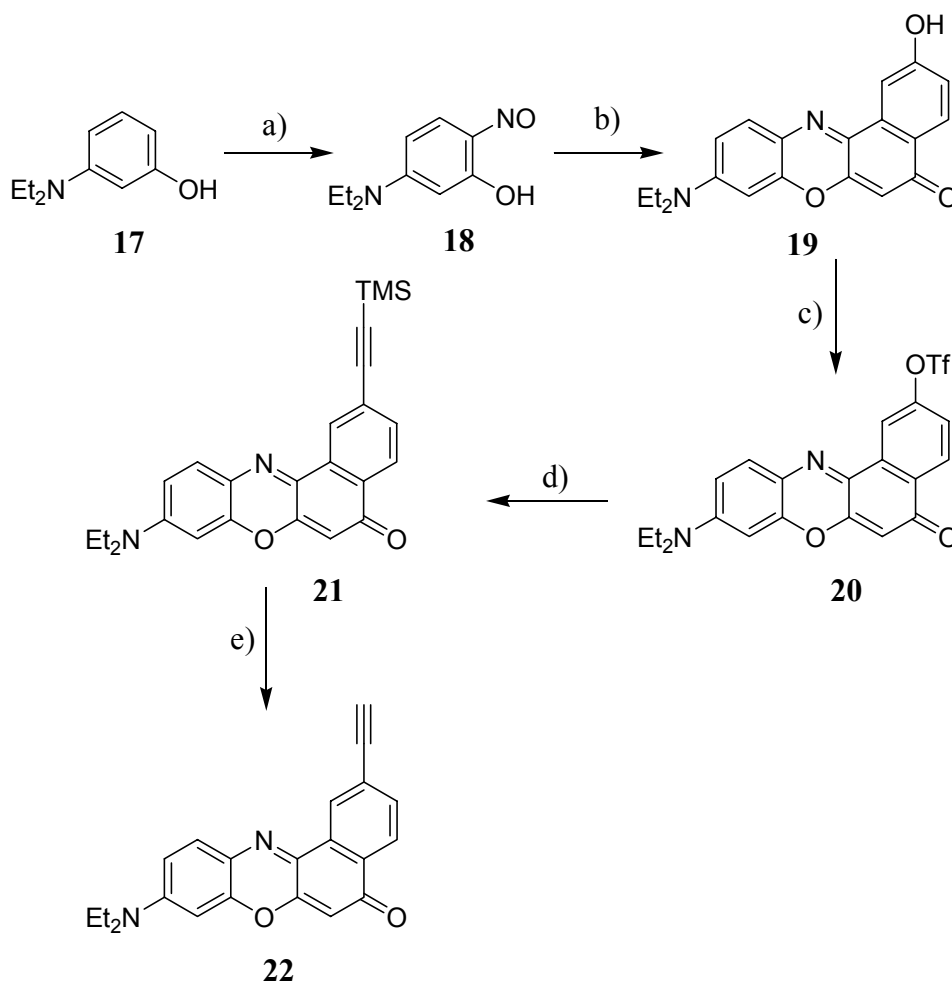


Scheme 2.4. Synthesis of 3-Ethynylperylene **16**. Reagents and conditions: a) NBS (1.0 eq.), DMF, r.t., 26 h, 94 %; b) $(\text{CH}_3)_3\text{SiCCH}$ (1.7 eq.), $\text{PdCl}_2(\text{PPh}_3)_2$ (10.0 mol-%), CuI (6.1 mol-%), DMF, NEt_3 , 85 °C, 20 h, 97 %; c) K_2CO_3 (3.8 eq.), MeOH, THF, r.t., 61 h, 97 %.

2.2.5. Synthesis of Ethynyl modified nile red

As depicted in Scheme 2.5, the 2-ethynyl derivative **22** of nile red was prepared in five steps, following the general outline.^[23] The synthesis was initiated by nitrosation of 3-diethylaminophenol **17** with isopentyl nitrite and gave 5-diethylamino-2-nitrosophenol hydrochloride **18** in very good yield. The 2-hydroxy derivative **19** was prepared by reaction of the nitrosophenol hydrochloride **18** and 1,6-dihydroxynaphthalene in boiling N,N-dimethylformamide in 73 % yield.

19 shows a strong red fluorescence and is therefore very easy to detect during flash chromatography. Gradient elution was our method of choice since it cultivates very accurate and narrow bands. At this point we foreclose that it was also possible to perform very fast separations and purifications using gradient elution for the following benzo[*a*]phenoxazine compounds **20**, **21** and **22** with use of flash chromatography or dry-vacuum flash chromatography.^[24] However, in the next step, the hydroxy group was converted to the trifluoromethanesulfonate ester **20** with a N-triflated amide as acylating reagent in 75 % yield. The 2-trimethylsilyl-protected ethynyl modified nile red **21** was then synthesized by Pd-catalyzed Sonogashira-Hagihara cross-coupling reaction of **20** and trimethylsilylethylene in heated DMF, where $\text{Pd}(\text{PPh}_3)_4$ was an effective catalyst. After flash chromatography, trituration in a tetrahydrofuran/diethyl ether mixture gave **21** as wine red crystals. Finally, **21** was smoothly silyl deprotected with TBAF in a mixture of dichloromethane and tetrahydrofuran within 10 minutes to yield **22** in 94 % yield.



Scheme 2.5. Synthesis of 2-ethynyl nile red **22**. Reagents and conditions: a) Isopentyl nitrite (1.0 eq.), HCl (5.0 eq.), 0 °C, 2 h, 95 %; b) 1,6-dihydroxynaphthalene (1.0 eq.), DMF, reflux, 4 h, 73 %; c) N-phenyl-bis(trifluoromethane sulphonamide) (2.4 eq.), NEt₃ (2.4 eq.), THF, r.t., 24 h, 75 %; d) (CH₃)₃SiCCH (1.6 eq.), Pd(PPh₃)₄ (3.5 mol-%), CuI (3.5 mol-%), DMF, NEt₃, 80 °C, 4 h, 79 %; e) Bu₄NF (2.0 eq.), CH₂Cl₂, THF, r.t., 10 min, 94 %.

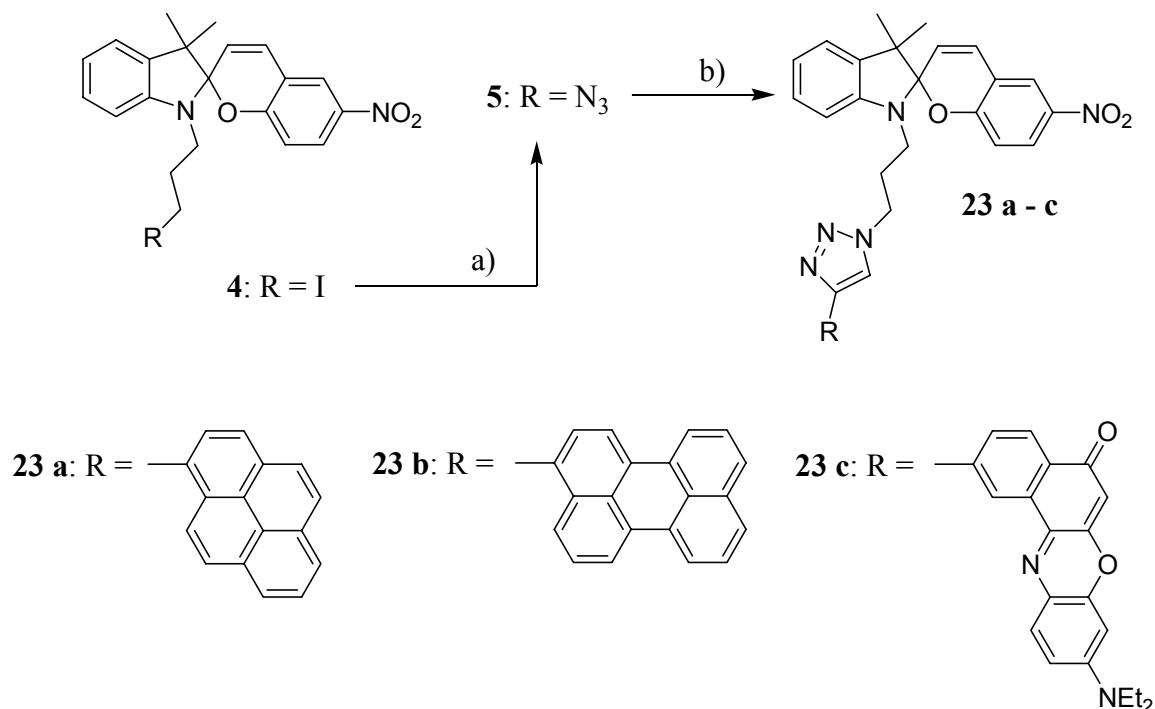
2.2.6. Click synthesis of molecular dyads

According to our outlined strategy and in order to synthesize the dyads we chose the click reaction (CuAAC) as a facile methodology for conjugation of spirobenzopyran azide with the previously synthesized ethynyl-bearing chromophores (**12**, **16** and **22**) and commercially available 1-ethynylpyrene.

For conjugation of the azide compound **5** and **12** we attempted click reactions using various mixtures of common polar and apolar solvents due to the very poor solubility of **12**. However, no reaction was observed and thus, we changed to run the click reaction in a “one-pot” manner that was recently reported to offer a straightforward methodology for the synthesis

and transformation of molecules containing terminal triple bonds.^[25] Using this approach we slowly added the K_2CO_3 solution in a mixture of water/methanol/tetrahydrofuran to the trimethylsilylacetylene pyrene **11** while maintaining click conditions, i.e. the spirobenzopyran azide **5**, TBTA and copper(II) salt and sodium ascorbate were also present in the mixture so that sequential deprotection and click reaction of pyrene with the azide could occur. Although the deprotection of the TMS groups was performed slowly, TLC did not show formation of the desired triazole with the azide but precipitation of **12**. However, this attempt was no avail and can be allocated to the very poor solubility of the deprotected pyrene compound.

Nevertheless, the spirobenzopyran azide **5** was reacted in a click reaction with compounds 1-ethynyl-pyrene, **16** and **22** to form the dyads **23 a**, **23 b** and **23 c**, respectively. Evidently, the demand of the copper(I)-catalyzed cycloaddition for better solubility of compounds proved true. The click reaction was performed according to a general procedure in a water/DMF mixture and afforded the three dyads **23 a-c**. Flash chromatography was utilized for purification of the dyads, with slow increase of polarity during gradient elution, and we were able to isolate conjugates **23a**, **23 b** and **23 c**, bearing pyrene, perylene and nile red as chromophores in excellent yields.



Scheme 2.6. Synthesis of photochromic dyads **23 a - 23 c**. Reagents and conditions: a) NaN_3 (4.1 eq.), DMF, r.t., 19 h, 83 %; b) $HC\equiv CR$, $CuSO_4$ (15-21 mol-%), Na ascorbate (29-40 mol-%), TBTA (5-7.5 mol-%), DMF, H_2O , r.t., 24 h, **23 a**: 91 %, **23 b**: 84 %, **23 c**: 91 %.

2.2.7. Optical properties of molecular dyads

The UV/Vis absorption of the spiropyran conjugates **23 a-c** (Figure 1) after irradiation at 590 nm display exclusively the spiropyran isomer and the additional chromophore by the characteristic bands at ~ 350 nm (pyrene in **23 a**), ~ 425 nm (perylene in **23 b**) or ~ 550 nm (nile red in **23 c**), respectively. After irradiation with UV light, the photogenerated extended π -system of the merocyanine forms of all three dyads show an additional absorption in the visible region at ~ 560 nm.

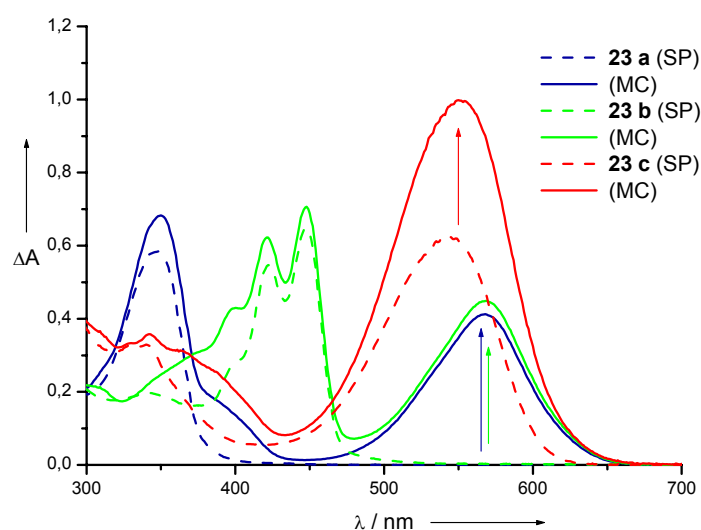


Figure 2.1. UV/Vis absorption spectra of **23 a-c** (20 μ M in MeCN) after irradiation at 312 nm (solid lines, MC), and after irradiation at 590 nm (dashed lines, SP).

If the dyads are excited at their characteristic wavelength (**23 a**: 355 nm, **23 b**: 413 nm, **23 c**: 545 nm) the fluorescence (Figure 2) is quenched by the merocyanine which is even more obvious if the data is corrected by the optical densities at the excitation wavelength. The latter result indicates an energy transfer process from the chromophore to the merocyanine which makes these switches interesting candidates for functional π -systems. The efficiency of this process increases from dyad **23 a** over **23 b** to **23 c** due to the enhanced spectral overlap. In **23 c**, however, the nile red can not be excited selectively because the merocyanine absorbs in the same range. Dyad **23 a** shows a small emission at ~ 650 nm probably due an exciplex.

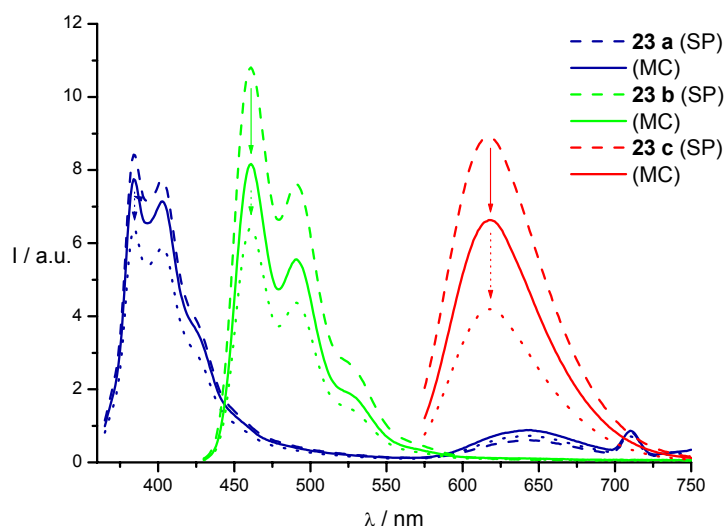


Figure 2.2. Fluorescence spectra of **23 a** (20 μM in MeCN) and **23 b-c** ($\sim 1 \mu\text{M}$ in MeCN) after irradiation at 312 nm (solid lines, MC), and after irradiation at 590 nm (dashed lines, SP), $\lambda_{\text{exc}} = 355 \text{ nm}$ (**23 a**), 413 nm (**23 b**), 545 nm (**23 c**). The dotted lines represent the spectra that are corrected by the differences in optical density at the excitation wavelength.

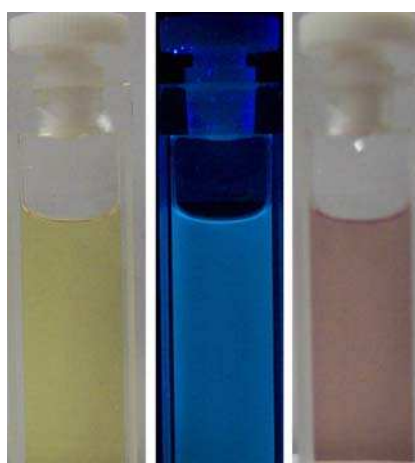


Image 2.1. Visual representation of photochromic switching in dyad **23 b**. After Vis irradiation, during UV irradiation, after UV irradiation (from left to right).

A useful extension for the improvement of spectral overlap is provided by new green-emitting fluorophores. These fluorescent dyes are currently being developed in our group and make meaningful units for the construction of additional dyads where fluorescence is even more strongly quenched by energy transfer. Thus, incorporated into DNA, these dyads serve as

valuable assemblies for photoswitching and manipulation of emission intensity and optical signals by light and can be used for clever biomedical applications.

2.3. Conclusion

In conclusion, a spirobenzopyran azide and fluorescent dyes bearing terminal alkynes groups have been prepared. With use of these building blocks, three novel photoswitchable fluorescent dyads **23 a-c** have been successfully synthesized using click chemistry as an elaborate and reasoned methodology. The photochromism was investigated using absorption and fluorescence spectroscopy. The dyads are composed of two units, integrating a spirobenzopyran photochrome and luminescent components (pyrene, perylene or nile red) in their molecular skeleton. The click reaction was used to conjugate the photochromic unit (azide) and the fluorophores (alkynes) by 1,4-regioselective formation of 1,2,3-triazoles.

The spectroscopic measurements show that by UV irradiation the spirobenzopyran unit of the dyads switch into their corresponding merocyanine form with appearance of its characteristic absorption band in the visible range. Fluorescence is quenched by the photoinduced merocyanine form for all three dyads and indicates an energy transfer process from the fluorophore unit to the merocyanine. The efficiency of this process increases from dyad **23 a** over **23 b** to **23 c** due to the enhanced spectral overlap.

In commemoration, since the switching of conformations and electronic states of organic molecules by external stimulations and input signals is of fundamental interest in matters of mechanism and potential applications, thus the results provide a novel synthetic strategy for engineering and creating new photochromic functional π -systems based on the concept of click chemistry. Meanwhile, this concept is followed up in the group of Prof. Decurtins for the construction of new materials and promises reasonable continuation of the project using photochromic spirobenzopyrans in a synergistic way with BDF chromophores.^[26]

2.4. Experimental Section

General

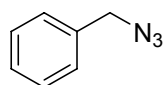
Conferrable details on reagents, solvents, reaction processing, chromatography, NMR, IR, UV/Vis and fluorescence spectroscopy and MS are registered in the general section of the

experimental part in Chapter 1. Compound **5** was prepared according to the synthetic procedure reported in the experimental section in Chapter 1.

Light sources

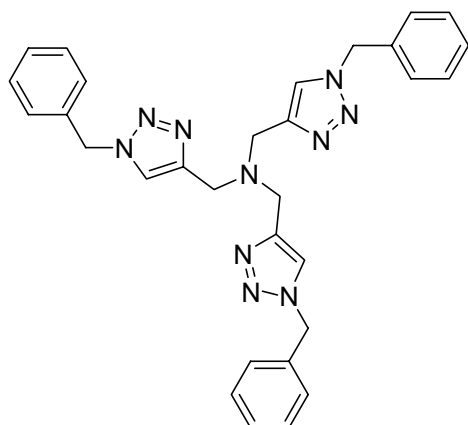
For irradiation experiments a UV hand-held lamp (Herolab, 6 W, $\lambda = 312$ nm) and a Luxeon III Star high-power LED ($\lambda = 590$ nm / amber) were used.

Benzylazide



A stock solution of sodium azide was prepared by stirring sodium azide (4.877 g, 75.02 mmol) in DMSO (158 mL) at room temperature for 24 hours. Following, freshly distilled benzylbromide (11.70 mL, 98.51 mmol) was added and the reaction mixture was stirred for 90 minutes. Water was added, the mixture was stirred for 30 minutes and extracted with Et₂O. The organic layer was washed with water (2 x) and brine (2 x), dried over anhydrous MgSO₄, the solvent was removed under reduced pressure and the residue was dried in vacuo over night to give a colorless oil. Finally, the product was purified by distillation to afford benzylazide as colorless oil (9.590 g, 96 %).

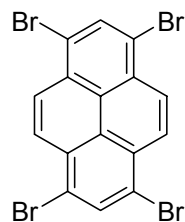
¹H NMR (300 MHz, CDCl₃) δ = 7.47-7.27 (m, 5 H), 4.34 (s, 2 H) - ¹³C NMR (75 MHz, CDCl₃) δ = 135.4 (C_{quat.}), 128.9 (+, CH), 128.4 (+, CH), 128.3 (+, CH), 54.8 (-, CH₂)

Tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine

A mixture of tripropargylamine (2.2 mL, 15.55 mmol) and freshly prepared benzylazide (6.45 mL, 51.62 mmol) were added to a mixture of CH₂Cl₂/H₂O (32 mL, 1:1). Under vigorous stirring, copper(II) sulphate (621 mg, 2.49 mmol), L-ascorbic acid (1.204 g, 6.84 mmol), water (2 mL) and an aqueous sodium hydroxide solution (6.84 mL, 6.88 mmol) were added. The bright yellow reaction mixture was stirred at room temperature for 14 hours. Following, CH₂Cl₂ (50 mL) and water (50 mL) were added. The aqueous layer was extracted again with CH₂Cl₂ (50 mL), the organic layers were pooled and dried over anhydrous MgSO₄. The product was subjected to gradient flash chromatography (CH₂Cl₂/MeOH 100:1 to 30:1). The solvents were removed in vacuo, MeOH was added and Et₂O was carefully layered on top to provide tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine as glistening white crystals (7.949 g, 96 %). R_f = 0.19 (CH₂Cl₂/MeOH 100:1)

¹H NMR (300 MHz, CDCl₃): δ = 7.65 (s, 3 H, CH-triazol), 7.37-7.30 (m, 9 H, H-Ar), 7.27-7.22 (m, 6 H, H-Ar), 5.49 (s, 6 H), 3.69 (s, 6 H) - ¹³C NMR (75 MHz, CDCl₃): δ = 144.3 (C_{quat.}), 134.8 (C_{quat.}), 129.1 (+, CH), 128.7 (+, CH), 128.0 (+, CH), 123.7 (+, CH), 54.1 (-, CH₂), 47.1 (-, CH₂) - MS (ESI): m/z (%): 531.2 (100) [MH⁺] - HRMS (EI-MS) calcd. for C₃₀H₃₀N₁₀ [M⁺]: 530.2655, found: 530.2654

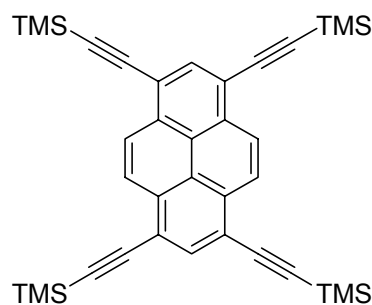
1,3,6,8-Tetrabromopyrene



To a solution of pyrene (7.263 g, 35.91 mmol) in nitrobenzene (170 mL), there was added bromine (7.82 mL, 152.62 mmol) over 15 minutes. The mixture was heated at 140 °C for 4 hours, stirred vigorously and slowly cooled to room temperature whereas spontaneous formation of a precipitate was observed. Following, an aqueous solution of sodium sulfite (11.90 mmol, 14 mL) was added and the mixture was stirred at room temperature for 15 minutes. The precipitate was filtered, washed with water (10 mL), methanol (40 mL) and dried in vacuo to yield 1,3,6,8-tetrabromopyrene as a yellow solid (17.192 g, 92 %).

MS (CI, 70 eV): m/z (%): 515.8 (63) [M^+], 517.9 (100) [M^+]

1,3,6,8-Tetrakis(2-(trimethylsilyl)ethynyl)pyrene

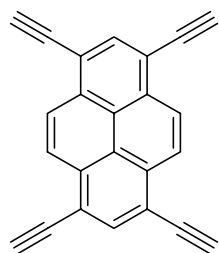


A Schlenk flask was charged under argon atmosphere with 1,3,6,8-tetrabromopyrene (339 mg, 0.655 mmol), $\text{PdCl}_2(\text{PPh}_3)_2$ (93.7 mg, 0.134 mmol), CuI (12.4 mg, 0.0651 mmol), triphenylphosphine (35 mg, 0.133 mmol), dry NEt_3 (6.5 mL) and dry DMF (6.5 mL) were added. The mixture was degassed via freeze-pump-thaw (3 cycles) and Ethynyltrimethylsilane (0.56 mL, 3.963 mmol) was added under argon atmosphere. The reaction mixture was heated at 70 °C for 20 hours and slowly cooled to room temperature. CH_2Cl_2 (5 mL) and water (5 mL) were added and the mixture was stirred for 10 minutes. Following, the mixture was

extracted with CH_2Cl_2 , washed with brine and water and the aqueous layer was extracted with CH_2Cl_2 . The organic layers were pooled and dried over anhydrous MgSO_4 , filtered and the solvent was evaporated under reduced pressure. The residue was dried in vacuo and the remaining solid was purified by flash chromatography on silica gel (Hexane) to give 1,3,6,8-tetrakis(2-(trimethylsilyl)ethynyl)pyrene as lustrous orange crystals (300 mg, 78 %). $R_f = 0.13$ (Hexane)

^1H NMR (300 MHz, CDCl_3): $\delta = 8.59$ (s, 4 H), 8.31 (s, 2 H), 0.39 (s, 36 H) - ^{13}C NMR (75 MHz, CDCl_3): $\delta = 134.4, 131.9, 126.9, 118.5, 102.7, 101.3, -0.1$ - MS (CI, NH_3): m/z (%) = 586.3 (100) $[\text{M}^+]$, 571.2 (11) $[\text{M}^+ - \text{CH}_3]$

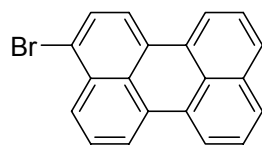
1,3,6,8-Tetraethynylpyrene



A flask was charged with 1,3,6,8-tetrakis(2-(trimethylsilyl)ethynyl)pyrene (203 mg, 0.346 mmol) and a mixture of THF/MeOH (1:1, 24 mL). To this blood-red suspension potassium carbonate (384 mg, 2.778 mmol) was added and the mixture was stirred vigorously in the dark at room temperature for 16 hours. The formed precipitate was filtered, washed with water (200 mL) and dried in vacuo. 1,3,6,8-tetraethynylpyrene was obtained as a yellow-brown solid (98 mg, 95 %).

IR (neat): ν [cm^{-1}] = 3277, 1599, 905

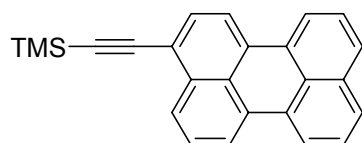
3-Bromoperylene



Perylene (518 mg, 2.05 mmol) was dissolved in dry N,N-Dimethylformamide (95 mL). After stirring for 15 minutes under nitrogen, a solution of N-Bromosuccinimide (365 mg, 2.05 mmol) in dry N,N-Dimethylformamide (20.5 mL) was injected through a syringe. The solution was stirred at room temperature for 26 h under a slow flow of nitrogen. Water (400 mL) was added and the solution was stirred for 1 hour at room temperature. The resulting precipitate was separated through suction filtration, rinsed with water, and dried under vacuum. The precipitate was then recrystallized from hexane to afford 3-Bromoperylene as yellow crystals (639 mg, 94 %).

^1H NMR (300 MHz, CDCl_3): δ = 8.27-8.10 (m, 4 H), 8.07 (d, 1 H, J = 8.0 Hz), 7.97 (d, 1 H, J = 8.0 Hz), 7.74-7.68 (m, 2 H), 7.59-7.46 (m, 3 H) - MS (EI, 70 eV): m/z (%): 330.0 (100) [M^+], 332.0 (84)

Trimethyl(2-(perylene-4-yl)ethynyl)silane

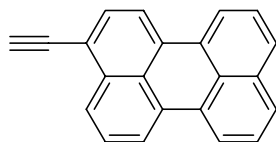


A small Schlenk tube with stirring bar was flushed with argon and charged with 3-Bromoperylene (104 mg, 0.312 mmol) and dry N,N-Dimethylformamide (3 mL). The solution was degassed via freeze-pump-thaw (3 cycles), $\text{PdCl}_2(\text{PPh}_3)_2$ (21.9 mg, 0.0312 mmol), CuI (3.6 mg, 0.0189 mmol) and NEt_3 (3 mL) were added under inert atmosphere. To the orange-yellow solution Ethynyltrimethylsilane (52.1 mg, 0.531 mmol) was added. The reaction was stirred at 85 °C for 20 hours, quenched with water and extracted with CH_2Cl_2 (3 x 20 mL). The combined organic phases were washed with brine, dried over anhydrous Na_2SO_4 , the solution was filtered and concentrated in vacuo. Purification was done by gradient flash

chromatography on silica gel (Hexane/CH₂Cl₂ 200:1 to 50:1) and Trimethyl(2-(perylene-4-yl)ethynyl)silane was isolated as lustrous yellow-orange crystals (106 mg, 97 %). $R_f = 0.18$ (Hexane/CH₂Cl₂ 100:1)

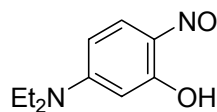
¹H NMR (300 MHz, CDCl₃): $\delta = 8.20$ (dd, 4 H, $J = 7.6, 15.2$ Hz), 8.10 (d, 1 H, $J = 7.9$ Hz), 7.73 - 7.65 (m, 3 H), 7.57 (t, 1 H, $J = 7.6$ Hz), 7.48 (dt, 2 H, $J = 1.7, 7.9$ Hz), 0.34 (s, 9 H) - MS (EI, 70 eV): m/z (%): 348.2 (100) [M^+], 349.2 (60)

3-Ethynylperylene



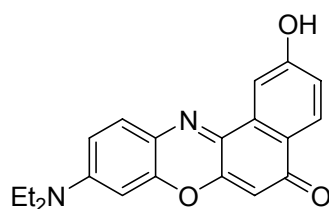
Trimethyl(2-(perylene-4-yl)ethynyl)silane (106 mg, 0.303 mmol) was dissolved in a mixture of MeOH (20 mL) and THF (15 mL) and finely grounded K₂CO₃ (157 mg, 1.136 mmol) was added. The reaction mixture was stirred at room temperature for 61 hours, then water (50 mL) and CH₂Cl₂ (50 mL) were added and the solution was stirred for 30 minutes. The mixture was extracted with CH₂Cl₂ (3 x 50 mL), washed with brine and dried over Na₂SO₄. The organic solvent was evaporated under reduced pressure and the residue was dried in vacuo to afford 81 mg of 3-Ethynylperylene as glistening yellow crystals (0.29347 mmol, 97 %).

¹H NMR (300 MHz, CDCl₃): $\delta = 8.18$ - 8.09 (m, 4 H), 8.02 (d, 1 H, $J = 7.9$ Hz), 7.67 - 7.62 (m, 3 H), 7.51 (t, 1 H, $J = 7.9$ Hz), 7.43 (dt, 2 H, $J = 2.5, 7.9$ Hz), 3.53 (s, 1 H) - IR (neat): ν [cm⁻¹] = 2924, 2361, 1738, 1375, 1217 - MS (EI, 70 eV): m/z (%): 276.1 (100) [M^+]

5-Diethylamino-2-nitrosophenol hydrochloride

3-Diethylaminophenol (4.125 g, 24.97 mmol) was dissolved in a 4 M HCl solution in dioxane (32.5 mL, 125 mmol), cooled to 0 °C and freshly distilled isopentyl nitrite (3.37 mL, 25.09 mmol) was added dropwise at 0 °C for 75 minutes and stirred for additional 45 minutes. Cold Et₂O (45 mL) was added to the mixture and the precipitate was filtered off, washed with dioxane (10 mL) and Et₂O (45 mL) to afford 5-Diethylamino-2-nitrosophenol hydrochloride as a pale brown solid (4.627 g, 95 %).

¹H NMR (300 MHz, MeOD): δ = 7.71 (d, 1 H, J = 10.4 Hz), 7.20 (d, 1 H, J = 10.4 Hz), 6.40 (d, 1 H, J = 2.4 Hz), 3.91 (qd, 4 H, J = 7.2, 25.8 Hz), 1.39 (t, 6 H, J = 7.2 Hz) - ¹³C NMR (75 MHz, MeOD) δ = 167.2, 164.1, 124.8, 121.0, 68.2, 23.0, 14.7, 13.1 - MS (ESI): m/z (%): 195.0 (100) [MH⁺], 236.0 (11) [MH⁺+MeCN]

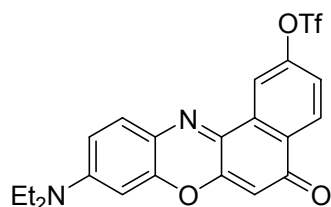
9-(diethylamino)-2-hydroxy-5H-benzo[*a*]phenoxazin-5-one

To a dry flask containing 5-Diethylamino-2-nitrosophenol hydrochloride (2.892 g, 12.57 mmol) in dry DMF (130 mL), a solution of 1,6-dihydroxynaphthalene (2.019 g, 12.61 mmol) in dry DMF (130 mL) was added. The reaction mixture was refluxed in the dark for 4 hours, slowly cooled to room temperature and DMF was removed under reduced pressure. The resulting dark solid was dissolved in EtOAc, washed with brine and water. The organic layers were pooled, concentrated to half volume and dried over anhydrous Na₂SO₄. The residue was dried in vacuo to constant weight and the remaining solid was purified by gradient flash chromatography on silica gel (Hexane/EtOAc 8:2 to 3:7) to give 9-(diethylamino)-2-hydroxy-

5H-benzo[*a*]phenoxazin-5-one as a dark olive-green solid (3.085 g, 73 %). $R_f = 0.60$ (Hexane/EtOAc 1:1)

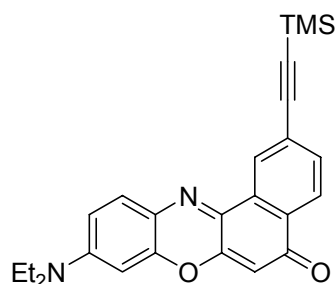
^1H NMR (300 MHz, DMSO- d_6): $\delta = 10.43$ (s, 1 H), 7.96 (d, 1 H, $J = 8.6$ Hz), 7.87 (d, 1 H, $J = 2.5$ Hz), 7.57 (d, 1 H, $J = 9.1$ Hz), 7.08 (dd, 1 H, $J = 2.5, 8.6$ Hz), 6.79 (dd, 1 H, $J = 2.7, 9.1$ Hz), 6.62 (d, 1 H, $J = 2.6$ Hz), 6.14 (s, 1 H), 3.48 (q, 4 H, $J = 7.0$ Hz), 1.15 (t, 6 H, $J = 6.9$ Hz) - MS (EI, 70 eV): m/z (%): 319.1 (100) [$\text{M}^+ - \text{CH}_3$], 334.2 (56) [M^+]

9-(diethylamino)-5-oxo-5H-benzo[*a*]phenoxazin-2-yl trifluoromethanesulfonate



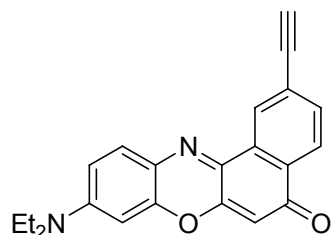
Under nitrogen atmosphere a flask was charged with 9-(diethylamino)-2-hydroxy-5H-benzo[*a*]phenoxazin-5-one (3.050 g, 9.13 mmol) and dry THF (250 mL). The solution was degassed and N-phenyl-bis(trifluoromethane sulphonamide) (8.114 g, 22.31 mmol) and dry NEt_3 (3.05 mL, 21.89 mmol) were added. The mixture was stirred in the dark at room temperature for 24 hours, diluted with CH_2Cl_2 , washed with water and dried over anhydrous MgSO_4 . The organic solvents were removed in vacuo and the crude product was purified by gradient flash chromatography on silica gel (Hexane/EtOAc 8:2 to 4:6) to give 9-(diethylamino)-5-oxo-5H-benzo[*a*]phenoxazin-2-yl trifluoromethanesulfonate as lustrous dark red crystals (3.200 g, 75 %). $R_f = 0.67$ (Hexane/EtOAc 1:1)

^1H NMR (300 MHz, CDCl_3): $\delta = 8.52$ (d, 1 H, $J = 2.5$ Hz), 8.40 (d, 1 H, $J = 8.8$ Hz), 7.63 (d, 1 H, $J = 9.2$ Hz), 7.48 (dd, 1 H, $J = 2.5, 8.7$ Hz), 6.71 (dd, 1 H, $J = 2.7, 9.2$ Hz), 6.47 (d, 1 H, $J = 2.7$ Hz), 6.44 (s, 1 H), 3.48 (q, 1 H, $J = 7.1$ Hz), 1.28 (t, 1 H, $J = 7.2$ Hz) - ^{13}C NMR (75 MHz, CDCl_3) $\delta = 182.2$ (C_{quat}), 152.9 (C_{quat}), 151.7 (C_{quat}), 151.5 (C_{quat}), 147.2 (C_{quat}), 137.2 (C_{quat}), 134.2 (C_{quat}), 131.8 (+, CH), 131.0 (C_{quat}), 128.7 (+, CH), 125.5 (C_{quat}), 122.2 (+, CH), 117.7 (C_{quat}), 116.4 (+, CH), 110.7 (+, CH), 105.2 (+, CH), 96.2 (+, CH), 45.3 (-, CH_2) 12.6 (+, CH_3) - MS (ESI): m/z (%): 466.9 (100) [MH^+]

9-(diethylamino)-2-((trimethylsilyl)ethynyl)-5H-benzo[*a*]phenoxazin-5-one

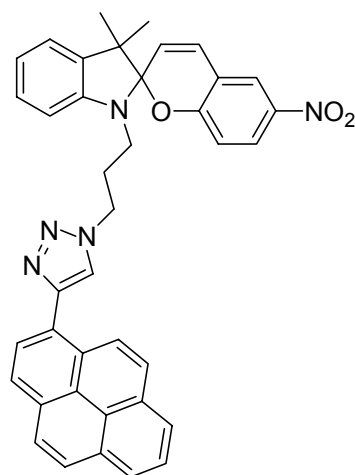
A Schlenk flask was charged under argon atmosphere with 9-(diethylamino)-5-oxo-5H-benzo[*a*]phenoxazin-2-yl trifluoromethanesulfonate (3.177 g, 6.82 mmol) and dry DMF (70 mL). Following, Pd(PPh₃)₄ (275.5 mg, 0.238 mmol), CuI (45.5 mg, 0.239 mmol), and dry NEt₃ (5.0 mL) were added. The mixture was degassed via freeze-pump-thaw (3 cycles) and Ethynyltrimethylsilane (1.50 mL, 10.61 mmol) was added under argon atmosphere. The reaction mixture was heated at 80 °C for 4 hours, slowly cooled to room temperature under vigorous stirring, CH₂Cl₂ (10 mL) and water (10 mL) were added and the mixture was stirred for 10 minutes. The mixture was extracted with CH₂Cl₂ (3 x 50 mL), washed with brine and water and the aqueous layer was extracted with CH₂Cl₂ (20 mL). The organic layers were combined and dried over anhydrous Na₂SO₄, filtered and the solvent was evaporated under reduced pressure. The residue was dried in vacuo over night and the remaining crude product was subjected to gradient flash chromatography on silica gel (Hexane/EtOAc 4:1 to 1:1). The purified product was triturated with a THF/Et₂O 1:1 mixture and 9-(diethylamino)-2-((trimethylsilyl)ethynyl)-5H-benzo[*a*]phenoxazin-5-one was obtained as a wine red crystals (2.230 g, 79 %). R_f = 0.52 (Hexane/EtOAc 1:1)

¹H NMR (300 MHz, CDCl₃): δ = 8.75 (d, 1 H, J = 1.3 Hz), 8.24 (d, 1 H, J = 8.1 Hz), 7.70 (dd, 1 H, J = 1.6, 8.2 Hz), 7.64 (d, 1 H, J = 9.1 Hz), 6.70 (dd, 1 H, J = 2.7, 9.1 Hz), 6.49 (d, 1 H, J = 2.7 Hz), 6.34 (s, 1 H), 3.49 (q, 4 H, J = 7.1 Hz), 1.27 (t, 6 H, J = 7.1 Hz), 0.30 (s, 9 H) - MS (ESI): m/z (%): 415.1 (100) [MH⁺]

9-(diethylamino)-2-ethynyl-5H-benzo[*a*]phenoxazin-5-one

To a solution of 9-(diethylamino)-2-((trimethylsilyl)ethynyl)-5H-benzo[*a*]phenoxazin-5-one (1.773 g, 4.28 mmol) in dry CH₂Cl₂ (24 mL), a freshly prepared solution of Bu₄NF in dry THF (8.56 mL, 8.56 mmol) was added. The reaction mixture was stirred at room temperature for 10 minutes, and water (25 mL) was added. The mixture was extracted with CH₂Cl₂ (2 x 20 mL) and the aqueous layer was extracted with EtOAc. The pooled organic layers were concentrated to half volume, dried over anhydrous Na₂SO₄ and the solvents were removed under reduced pressure. Purification was performed by gradient flash chromatography on silica gel (Hexane/EtOAc 2:1 to 1:1), followed by trituration with a Hexane/Et₂O mixture over night to yield 9-(diethylamino)-2-ethynyl-5H-benzo[*a*]phenoxazin-5-one as beautiful purple red crystals (1.383 g, 94 %). R_f = 0.42 (Hexane/EtOAc 1:1)

¹H NMR (400 MHz, CDCl₃): δ = 8.80 (d, 1 H, J = 1.4 Hz), 8.27 (d, 1 H, J = 8.2 Hz), 7.72 (dd, 1 H, J = 1.6, 8.1 Hz), 7.57 (dd, 1 H, J = 1.4, 2.9 Hz), 6.70 (dd, 1 H, J = 2.7, 9.1 Hz), 6.49 (d, 1 H, J = 2.7 Hz), 6.40 (s, 1 H), 3.49 (q, 1 H, J = 7.1 Hz), 3.26 (s, 1 H), 1.28 (t, 6 H, J = 7.1 Hz) - IR (neat): ν [cm⁻¹] = 2970, 2359, 1738, 1366, 1217 - MS (ESI): m/z (%): 343.1 (100) [MH⁺]

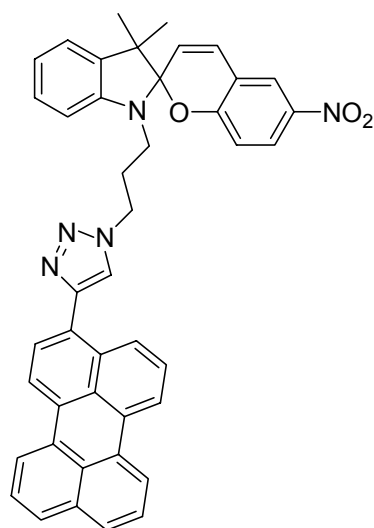
1-(1',3'-dihydro-3'3'-dimethyl-6-nitro-spiro[2H-1-benzopyran-2,2'[2H]indole]-1'-propyl-1H-[1,2,3]triazol-4-yl)-pyrene

To a solution of 2-(3',3'-Dimethyl-6-nitro-3'H-spiro[chromene-2,2'-indol]-1'-yl)-1-(3-Azidopropane) (182 mg, 0.465 mmol) in a 3:1 mixture (v/v) of DMF and water (24 mL), 1-Ethynylpyrene (105 mg, 0.464 mmol), Copper(II) sulphate pentahydrate (17 mg, 0.068 mmol), Tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine (12 mg, 0.023 mmol) and (+)-Sodium L-ascorbate (27 mg, 0.136 mmol) were added. The mixture was stirred at room temperature until complete consumption of the pyrene (~ 24 hours), diluted with EtOAc and washed with brine and water. The aqueous phase was extracted with CH₂Cl₂, the organic layers were combined and dried over anhydrous Na₂SO₄. The solvents were removed under reduced pressure and the crude product was purified by gradient flash chromatography on silica gel (CH₂Cl₂/MeOH 200:1 to 100:1) to afford 1-(1',3'-dihydro-3'3'-dimethyl-6-nitro-spiro[2H-1-benzopyran-2,2'[2H]indole]-1'-propyl-1H-[1,2,3]triazol-4-yl)-pyrene as a pale green foam (262 mg, 91 %). *R*_f = 0.17 (CH₂Cl₂/MeOH 200:1)

¹H NMR (600 MHz, CDCl₃): δ = 8.64 (d, 1 H, *J* = 9.2 Hz, H-Ar), 8.23-8.19 (m, 3 H, H-Ar), 8.14 (d, 1 H, *J* = 7.9 Hz, H-Ar), 8.10 (q, 3 H, *J* = 8.7 Hz, H-Ar), 8.03 (t, 1 H, *J* = 7.6 Hz, H-Ar), 7.98 (dd, 1 H, *J* = 2.7, 9.0 Hz, H-Ar), 7.85 (d, 1 H, *J* = 2.7 Hz, H-Ar), 7.83 (s, 1 H, triazol), 7.21 (dt, 1 H, *J* = 1.2, 7.7 Hz, H-Ar), 7.11 (dd, 1 H, *J* = 0.9, 7.2 Hz, H-Ar), 6.92 (t, 1 H, *J* = 7.4 Hz, H-Ar), 6.81 (d, 1 H, *J* = 10.3 Hz, H-Ar), 6.74 (d, 1 H, *J* = 9.0 Hz, H-Ar), 6.58 (d, 1 H, *J* = 7.7 Hz, H-Ar), 5.83 (d, 1 H, *J* = 10.3 Hz, H-Ar), 4.61-4.50 (m, 2 H, CH₂-propyl), 3.43-3.36 (m, 1 H, CH₂-propyl), 3.36-3.29 (m, 1 H, CH₂-propyl), 2.52-2.42 (m, 1 H, CH₂-propyl), 2.41-2.30 (m, 1 H, CH₂-propyl), 1.27 (s, 3 H, CH₃), 1.18 (s, 3 H, CH₃) - ¹³C NMR

(150 MHz, CDCl₃): δ = 159.2 (C_{quat.}), 147.7 (C_{quat.}), 146.6 (C_{quat.}), 141.0 (C_{quat.}), 136.1 (C_{quat.}), 131.4 (C_{quat.}), 130.8 (C_{quat.}), 128.5 (+, CH), 128.5 (C_{quat.}), 128.3 (+, CH), 127.9 (+, CH), 127.9 (+, CH), 127.3 (+, CH), 127.0 (+, CH), 126.1 (+, CH), 125.9 (+, CH), 125.5 (+, CH, triazol), 125.2 (+, CH), 125.1 (C_{quat.}), 125.0 (C_{quat.}), 124.9 (+, CH), 124.7 (C_{quat.}), 124.6 (+, CH), 122.7 (+, CH), 122.5 (+, CH), 122.0 (+, CH), 121.5 (+, CH), 120.0 (+, CH), 118.3 (C_{quat.}), 115.4 (+, CH), 106.7 (+, CH), 106.7 (C_{quat.}), 53.4 (C_{quat.}), 52.6 (C_{quat.}), 48.1 (-, CH₂), 40.8 (-, CH₂), 29.1 (-, CH₂), 25.9 (+, CH₃), 19.9 (+, CH₃) - HRMS (EI-MS) calcd. for C₃₉H₃₁N₅O₃ [M⁺]: 617.2427, found: 617.2417

3-(1',3'-dihydro-3'3'-dimethyl-6-nitro-spiro[2H-1-benzopyran-2,2'[2H]indole]-1'-propyl-1H-[1,2,3]triazol-4-yl)-perylene

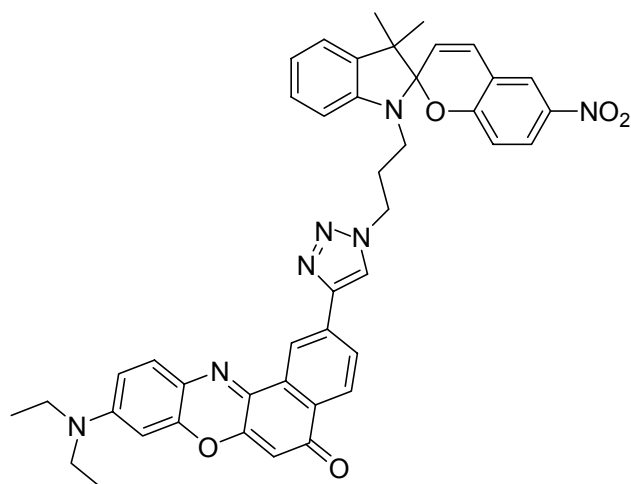


3-Ethynylperylene (56 mg, 0.203 mmol) and 2-(3',3'-Dimethyl-6-nitro-3'H-spiro[chromene-2,2'-indol]-1'-yl)-1-(3-Azidopropane) (76 mg, 0.208 mmol) were dissolved in a 3:1 mixture (v/v) of DMF and water (10 mL). (+)-Sodium L-ascorbate (14 mg, 0.071 mmol), Tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine (7 mg, 0.013 mmol) and Copper(II) sulphate pentahydrate (8 mg, 0.032 mmol), were added. The mixture was stirred at room temperature for 24 hours, diluted with CH₂Cl₂, washed with brine and water. The aqueous phase was extracted with CH₂Cl₂, the organic layers were combined and dried over anhydrous Na₂SO₄. The solvent was evaporated and the remaining solid was purified by gradient flash chromatography on silica gel (CH₂Cl₂/MeOH 1000:1 to 20:1) to yield 3-(1',3'-dihydro-3'3'-

dimethyl-6-nitro-spiro[2H-1-benzopyran-2,2'[2H]indole]-1'-propyl -1H-[1,2,3]triazol-4-yl)-perylene as a lustrous yellow solid (123 mg, 91 %). $R_f = 0.48$ (CH_2Cl_2)

^1H NMR (600 MHz, CD_2Cl_2): $\delta = 8.30$ -8.25 (m, 5 H, H-Ar), 7.99 (dd, 1 H, $J = 2.7, 9.0$ Hz, H-Ar), 7.91 (d, 1 H, $J = 2.7$ Hz, H-Ar), 7.79 (s, 1 H, triazol), 7.74 (dd, 2 H, $J = 4.4, 8.0$ Hz, H-Ar), 7.64 (d, 1 H, $J = 7.8$ Hz, H-Ar), 7.55-7.63 (m, 3 H, H-Ar), 7.18 (dt, 1 H, $J = 1.1, 7.7$ Hz, H-Ar), 7.11 (d, 1 H, $J = 7.2$ Hz, H-Ar), 6.89 (dd, 2 H, $J = 4.4, 8.8$ Hz, H-Ar), 6.77 (d, 1 H, $J = 8.9$ Hz, H-Ar), 6.57 (d, 1 H, $J = 7.8$ Hz, H-Ar), 5.88 (d, 1 H, $J = 10.3$ Hz, H-Ar), 4.58-4.48 (m, 2 H, CH_2 -propyl), 3.42-3.35 (m, 1 H, CH_2 -propyl), 3.34-3.27 (m, 1 H, CH_2 -propyl), 2.48-2.40 (m, 1 H, CH_2 -propyl), 2.36-2.29 (m, 1 H, CH_2 -propyl), 1.27 (s, 3 H, CH_3), 1.18 (s, 3 H, CH_3) - ^{13}C NMR (150 MHz, CD_2Cl_2): $\delta = 159.6$ ($\text{C}_{\text{quat.}}$), 147.2 ($\text{C}_{\text{quat.}}$), 147.1 ($\text{C}_{\text{quat.}}$), 141.5 ($\text{C}_{\text{quat.}}$), 136.6 ($\text{C}_{\text{quat.}}$), 135.1 ($\text{C}_{\text{quat.}}$), 132.7 ($\text{C}_{\text{quat.}}$), 132.0 ($\text{C}_{\text{quat.}}$), 131.8 ($\text{C}_{\text{quat.}}$), 131.5 ($\text{C}_{\text{quat.}}$), 131.2 ($\text{C}_{\text{quat.}}$), 129.5 ($\text{C}_{\text{quat.}}$), 128.9 (+, CH), 128.8 ($\text{C}_{\text{quat.}}$), 128.5 (+, CH), 128.3 (+, CH), 128.2 (+, CH), 128.1 (+, CH), 128.1 ($\text{C}_{\text{quat.}}$), 127.4 (+, CH), 127.1 (+, CH), 127.1 (+, CH), 126.2 (+, CH), 125.8 (+, CH), 123.1 (+, CH), 122.9 (+, CH, triazol), 122.3 (+, CH), 121.9 (+, CH), 121.1 (+, CH), 121.0 (+, CH), 121.0 (+, CH), 120.3 (+, CH), 120.2 (+, CH), 119.0 ($\text{C}_{\text{quat.}}$), 115.8 (+, CH), 107.3 (+, CH), 107.1 ($\text{C}_{\text{quat.}}$), 48.6 (-, CH_2), 41.2 (-, CH_2), 30.1 ($\text{C}_{\text{quat.}}$), 29.5 (-, CH_2), 26.1 (+, CH_3), 19.9 (+, CH_3) - HRMS (EI-MS) calcd. for $\text{C}_{43}\text{H}_{33}\text{N}_5\text{O}_3$ [M^+]: 667.2589, found: 667.2589

2-(1',3'-dihydro-3'3'-dimethyl-6-nitro-spiro[2H-1-benzopyran-2,2'[2H]indole]-1'-propyl -1H-[1,2,3]triazol-4-yl)-9-(Diethylamino)-5H-benzo[*a*]phenoxazin-5-one



9-(Diethylamino)-2ethynyl-5H-benzo[*a*]phenoxazin-5-one (18 mg, 0.053 mmol) and 2-(3',3'-Dimethyl-6-nitro-3'H-spiro[chromene-2,2'-indol]-1'-yl)-1-(3-Azidopropane) (27 mg, 0.069 mmol) were dissolved in a 3:1 mixture (v/v) of DMF and water (6 mL). Copper(II) sulphate pentahydrate (2.6 mg, 0.011 mmol), (+)-Sodium L-ascorbate (4.2 mg, 0.021 mmol), and Tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine (2.0 mg, 0.004 mmol) were added. The mixture was stirred at room temperature for 24 hours, diluted with EtOAc, washed with brine and water (2 x). The organic layers were combined and dried over anhydrous MgSO₄. The solvent was removed under reduced pressure and the crude product was purified by gradient flash chromatography on silica gel (CH₂Cl₂/MeOH 300:1 to 70:1) to yield 2-(1',3'-dihydro-3'3'-dimethyl-6-nitro-spiro[2H-1-benzopyran-2,2'[2H]indole]-1'-propyl -1H-[1,2,3]triazol-4-yl)-9-(Diethylamino)-5H-benzo[*a*]phenoxazin-5-one as a pink solid (32.3 mg, 84 %) *R*_f = 0.15 (CH₂Cl₂/MeOH 200:1)

¹H NMR (600 MHz, CDCl₃): δ = 8.99 (s, 1 H, triazol), 8.35 (d, 1 H, *J* = 8.2 Hz, H-Ar), 8.08 (d, 1 H, *J* = 8.2 Hz, H-Ar), 7.99 (dd, 1 H, *J* = 2.7, 9.0 Hz, H-Ar), 7.94-7.89 (m, 2 H, H-Ar), 7.62 (d, 1 H, *J* = 9.0 Hz, H-Ar), 7.20 (t, 1 H, *J* = 7.6 Hz, H-Ar), 7.10 (d, 1 H, *J* = 7.1 Hz, H-Ar), 6.89 (dd, 2 H, *J* = 8.8, 14.6 Hz, H-Ar), 6.74 (d, 1 H, *J* = 8.9 Hz, H-Ar), 6.69 (dd, 1 H, *J* = 2.4, 9.0 Hz, H-Ar), 6.55 (d, 1 H, *J* = 7.8 Hz, H-Ar), 6.48 (d, 1 H, *J* = 2.3 Hz, H-Ar), 6.39 (s, 1 H, CH-C=O), 5.84 (d, 1 H, *J* = 10.3 Hz, H-Ar), 4.55-4.45 (m, 2 H, CH₂-propyl), 3.48 (q, 4 H, *J* = 7.0 Hz, 2 x CH₂-CH₃), 3.40-3.26 (m, 2 H, CH₂-propyl), 2.47-2.38 (m, 1 H, CH₂-propyl), 2.37-2.27 (m, 1 H, CH₂-propyl), 1.29 (s, 3 H, CH₃), 1.26 (d, 6 H, *J* = 6.3 Hz, 2 x CH₂-CH₃),

1.19 (s, 3 H, CH₃) - ¹³C NMR (150 MHz, CDCl₃): δ = 183.3 (C_{quat.}), 159.2 (C_{quat.}), 152.3 (C_{quat.}), 150.9 (C_{quat.}), 147.3 (C_{quat.}), 146.9 (C_{quat.}), 146.5 (C_{quat.}), 141.1 (C_{quat.}), 139.5 (C_{quat.}), 136.0 (C_{quat.}), 133.2 (C_{quat.}), 132.6 (C_{quat.}), 131.2 (CH), 131.2 (C_{quat.}), 128.5 (CH), 127.9 (CH), 126.8 (CH), 126.6 (CH), 126.0 (CH), 125.0 (C_{quat.}), 122.8 (CH), 122.0 (CH), 121.5 (CH), 120.6 (CH, triazol), 120.5 (CH), 120.1 (CH), 118.3 (C_{quat.}), 115.5 (CH), 109.9 (CH), 106.7 (CH), 106.7 (C_{quat.}), 105.8 (CH-C=O), 96.3 (CH), 52.7 (C_{quat.}), 48.2 (CH₂-propyl), 45.1 (CH₂-CH₃), 40.8 (CH₂-propyl), 29.7 (CH₂-CH₃), 29.3 (CH₂-propyl), 25.9 (CH₃), 19.9 (CH₃), 14.1 (C_{quat.}), 12.6 (C_{quat.}) - HRMS (LSI-MS) calcd. for C₄₃H₄₀N₇O₅ [MH⁺]: 734.3091, found: 734.3086

2.5. References

- [1] a)V. V. Rostovtsev, L. G. Green, V. V. Fokin, K. B. Sharpless, *Angew. Chem., Int. Ed.* **2002**, *41*, 2596; b)C. W. Tornoe, C. Christensen, M. Meldal, *J. Org. Chem.* **2002**, *67*, 3057.
- [2] a)H. C. Kolb, M. G. Finn, K. B. Sharpless, *Angew. Chem. Int. Ed. Engl.* **2001**, *40*, 2004; b)B. H. Lipshutz, B. R. Taft, *Angew. Chem. Int. Ed. Engl.* **2006**, *45*, 8235.
- [3] P. Ball, *Chemistry World* **2007**, *4*, 46.
- [4] T. R. Chan, R. Hilgraf, K. B. Sharpless, V. V. Fokin, *Org. Lett.* **2004**, *6*, 2853.
- [5] F. Himo, T. Lovell, R. Hilgraf, V. V. Rostovtsev, L. Noodleman, K. B. Sharpless, V. V. Fokin, *J. Am. Chem. Soc.* **2005**, *127*, 210.
- [6] a)C. Spiteri, J. E. Moses, *Angew. Chem. Int. Ed. Engl.* **2010**, *49*, 31; b)V. O. Rodionov, V. V. Fokin, M. G. Finn, *Angew. Chem. Int. Ed. Engl.* **2005**, *117*, 2250; c)V. D. Bock, H. Hiemstra, J. H. van Maarseveen, *Eur. J. Org. Chem.* **2005**, 51.
- [7] W. H. Binder, R. Sachsenhofer, *Macromol. Rapid Commun.* **2007**, *28*, 15.
- [8] B. Füger, *Synlett* **2009**, 848.
- [9] P. M. E. Gramlich, C. T. Wirges, A. Manetto, T. Carell, *Angew. Chem., Int. Ed.* **2008**, *47*, 8350.
- [10] a)J. F. Billing, U. J. Nilsson, *J. Org. Chem.* **2005**, *70*, 4847; b)S. Hotha, R. I. Anegundi, A. A. Natu, *Tetrahedron Lett.* **2005**, *46*, 4585; c)S. Chandrasekhar, C. L. Rao, C. Nagesh, C. R. Reddy, B. Sridhar, *Tetrahedron Lett.* **2007**, *48*, 5869.

- [11] a)T. S. Seo, Z. Li, H. Ruparel, J. Ju, *J. Org. Chem.* **2003**, 68, 609; b)S. Berndt, N. Herzig, P. Kele, D. Lachmann, X. Li, O. S. Wolfbeis, H.-A. Wagenknecht, *Bioconjugate Chem.* **2009**, 20, 558.
- [12] a)C. Y. Jao, A. Salic, *Proc. Natl. Acad. Sci. U. S. A.* **2008**, 105, 15779; b)A. Salic, T. J. Mitchison, *Proc. Natl. Acad. Sci. U. S. A.* **2008**, 105, 2415.
- [13] D. D. Díaz, S. Punna, P. Holzer, A. K. McPherson, K. B. Sharpless, V. V. Fokin, M. G. Finn, *Polym. Sci., Part A* **2004**, 42, 4392.
- [14] S. Díez-González, A. Correa, L. Cavallo, S. P. Nolan, *Chem. Eur. J.* **2006**, 12, 7558.
- [15] X. C. Li Zhang, Peng Xue, Herman H. Y. Sun, Ian D. Williams, K. Barry Sharpless, Valery V. Fokin, and Guochen Jia, *J. Am. Chem. Soc.* **2005**, 127, 15998.
- [16] a)I. Yildiz, E. Deniz, F. R. Raymo, *Chem. Soc. Rev.* **2009**, 38, 1859; b)J. Cusido, E. Deniz, F. M. Raymo, *Eur. J. Org. Chem.* **2009**, 2031.
- [17] Q. Zhang, Z. Ning, Y. Yan, S. Qian, H. Tian, *Macromol. Rapid Commun.* **2008**, 29, 193.
- [18] a)S. G. Alvarez, M. T. Alvarez, *Synthesis* **1997**, 413; b)E. Lieber, T. S. Chao, C. N. R. Rao, *J. Org. Chem.* **1957**, 22, 238.
- [19] B.-Y. Lee, S. R. Park, H. B. Jeon, K. S. Kim, *Tetrahedron Lett.* **2006**, 47, 5105.
- [20] G. Venkataramana, S. Sankararaman, *Eur. J. Org. Chem.* **2005**, 4162.
- [21] V. L. Andronova, M. V. Skorobogaty, E. V. Manasova, Y. A. Berlin, V. A. Korshun, G. A. Galegov, *Bioorg. Khim* **2003**, 29, 262.
- [22] a)H. Maeda, Y. Nanai, K. Mizuno, J. Chiba, S. Takeshima, M. Inouye, *J. Org. Chem.* **2007**, 72, 8990; b)R. H. Mitchell, Y.-H. Lai, R. V. Williams, *J. Org. Chem.* **1979**, 44, 4733.
- [23] a)S. L. Black, W. A. Stanley, F. V. Filipp, M. Bhairo, A. Verma, O. Wichmann, M. Sattler, M. Wilmanns, C. Schultz, *Bioorg. Med. Chem.* **2008**, 16, 1162; b)M. S. J. Briggs, I. Bruce, J. N. Miller, C. J. Moody, A. C. Simmonds, E. Swann, *J. Chem. Soc., Perkin Trans. I* **1997**, 1051; c)K. Tainaka, Y. Fujiwara, A. Okamoto, *NASS* **2005**, 155; d)J. Han, J. Jose, E. Mei, K. Burgess, *Angew. Chem., Int. Ed.* **2007**, 46, 1684.
- [24] a)J. Leonard, B. Lygo, G. Procter, *Advanced practical organic chemistry* **1998**, Taylor & Francis CRC Press; b)L. M. Harwood, *Aldrichimica Acta* **1985**, 18, 25; c)D. S. Pedersen, C. Rosenbohm, *Synthesis* **2001**, 16, 2431.
- [25] K. Lőrincz, P. Kele, Z. Novák, *Synthesis* **2009**, 3527.
- [26] C. Yi, C. Blum, M. Lehmann, S. Keller, S.-X. Liu, G. Frei, A. Neels, J. Hauser, S. Schrüch, S. Decurtins, *J. Org. Chem.* **2010**, 75, 3350.

Chapter 3:

Synthesis of spirobenzopyran building blocks for DNA synthesis and internal covalent modifications

3.1. Introduction

Among the structurally miscellaneous photochromic compounds that were prepared and characterized,^[1] diazobenzenes and spirobenzopyrans are the most prominent representatives for photoswitching of biopolymers.^[2] However, mainly diazobenzene derivatives have been extensively studied in peptides to switch folding and conformation,^[3] and as artificial and photoswitchable bases in nucleic acids with commercial success.^[4] The *cis-trans* isomerization is switched by irradiation with light and the azobenzene moieties as part of the nucleic acids can reversibly control the dissociation and formation of a DNA duplex, photomodulate the DNA polymerase reaction, and the DNA triplex formation.^[3-4, 5] General aspects about these switches are discussed in Chapter 1. However, photoregulation of hybridization can also be accomplished irreversibly by introduction of caging systems into the DNA,^[2a, 6] or reversibly controlled upon *cis-trans* photoisomerization with fluorescence switching readouts of other photochromic nucleosides like ^{8FV}G.^[7]

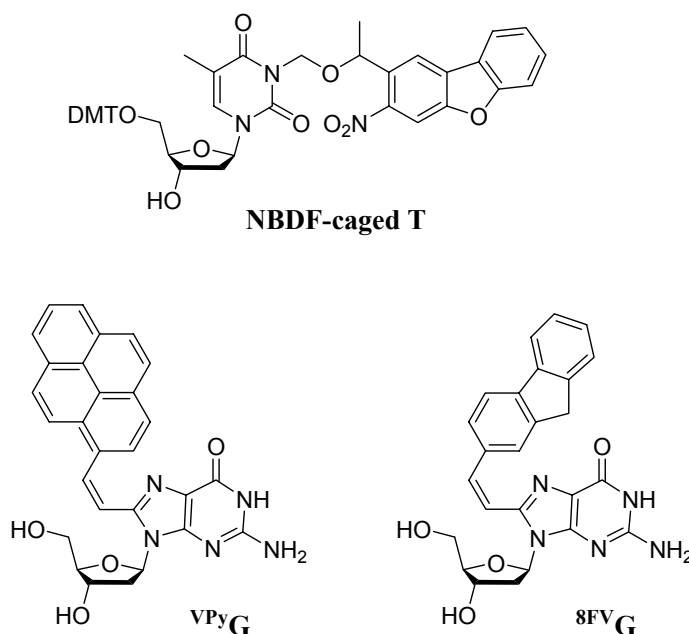


Figure 1.1. DMT-protected T, equipped with NDBF caging group^[6b], fluorescence switching nucleosides ^{VPy}G^[7c] and ^{8FV}G.^[7a]

Though, for reversible systems like spirobenzopyrans, the ring opening of the spirobenzopyrans to the open merocyanine does not only involve a significant structural change from nonplanar to a planar structure but also a large polarity change, in contrast to diazobenzenes.^[8] This is an important option with respect to nucleic acids because it can be assumed that the ring-closed spirobenzopyran form of these photoswitches is not able to insert into the base stack whereas the open and planar merocyanine form could potentially intercalate. This presumption was experimentally reinforced by photochromic spirobenzopyrans that were designed as noncovalent DNA and RNA binders.^[9] The strong ground state interaction that was only observed between the merocyanine form and the DNA bases induces a significant CD signal, i.e. indicating intercalation of the open merocyanine form into the DNA.^[9b, 10]

Although, amino acids,^[11] peptides,^[12] and proteins^[13] have been conjugated with spirobenzopyrans in order to endow these biomolecules with the optical switch. However, there are only two reports in the literature about the covalent linking of a spirobenzopyran to nucleic acids.^[14]

Herein, two new alternative synthetic routes to modify oligonucleotides covalently with spirobenzopyrans are reported: for the first route a spirobenzopyran phosphoramidite was synthesized as a DNA building block; the second route applies a postsynthetic click-type ligation strategy. As a surrogate for the 2'-deoxyribose moiety in DNA, acyclic linker systems have been established over the last years,^[15] and it turned out to be useful to replace the naturally occurring 2'-deoxyribofuranoside moiety by (S)-3-amino-1,2-propanediol as an acyclic linker.^[15b, 16]

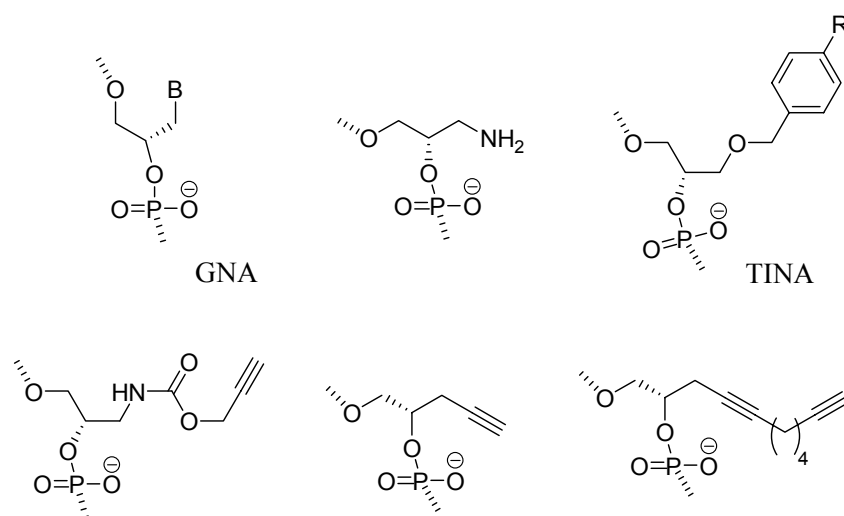


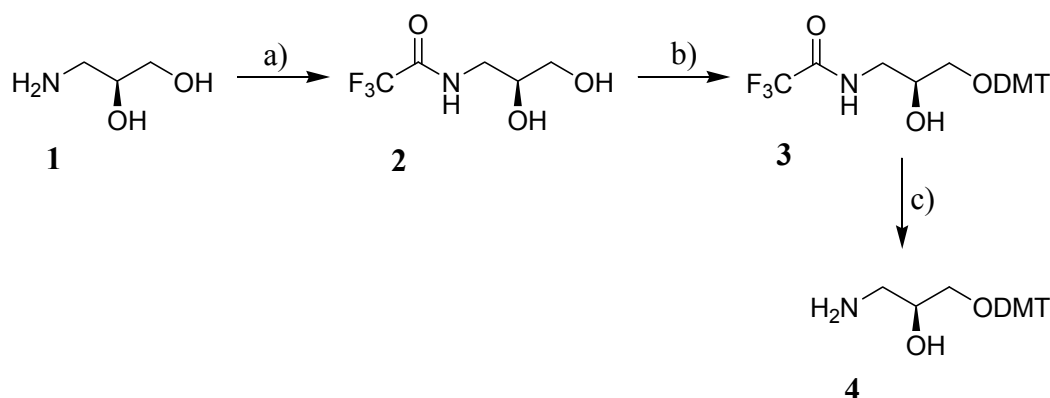
Figure 1.2. Typical acyclic building blocks.^[17]

The replacement of the labile glycosidic bond between the 2'-deoxyribofuranoside and a chromophore by using acyclic linker systems eludes stability issues that arise with glycosylamines.^[17a] Similar acyclic linker systems have been prepared, e.g. based on glycol nucleic acids (GNA)^[17c, 18] or twisted intercalating nucleic acids (TINA).^[17d] Meanwhile, these linker systems have been connotatively expanded and refined towards sophisticated bioorthogonal labeling, by endowing them with alkyne functionalities for further postsynthetic modifications.^[17b, 17e, 19] Further aspects on postsynthetic strategies are described in Chapter 4.

3.2. Results and Discussion

3.2.1. Synthesis of acyclic linker

The acyclic linker that has been used contains a primary and secondary hydroxy function, to serve as platform for the functionalization for the DNA building block chemistry, and also an additional primary amino group to attach the desired modifications. Synthesis of the building block **4** was carried out based on the acyclic linker that was established in our group.^[15b, 16] The synthesis of **4** consists of three steps (Scheme 2.1). First, the amino-group of (S)-3-amino-1,2-propanediol **1** was reacted with an excess of methyl trifluoroacetate at room temperature for 24 hours to afford **2** in 90 % yield. Second, the primary hydroxy-group was tritylated, to serve as the 5'-position during later DNA synthesis. Since it is crucial to prevent hydrolysis of DMT chloride already upon start of the reaction, it should be stressed out, that freshly dried solvents were used for the reaction and thus, **3** was obtained in 93 % yield. The final step for the synthesis of **4** involved the deprotection to regain the primary amino-function. The reaction was performed by stirring **3** in aqueous ammonia solution in a mixture of methanol and tetrahydrofuran and workup by extraction with dichloromethane. **4** was then isolated as a colorless fuzzy foam in 89 % yield.

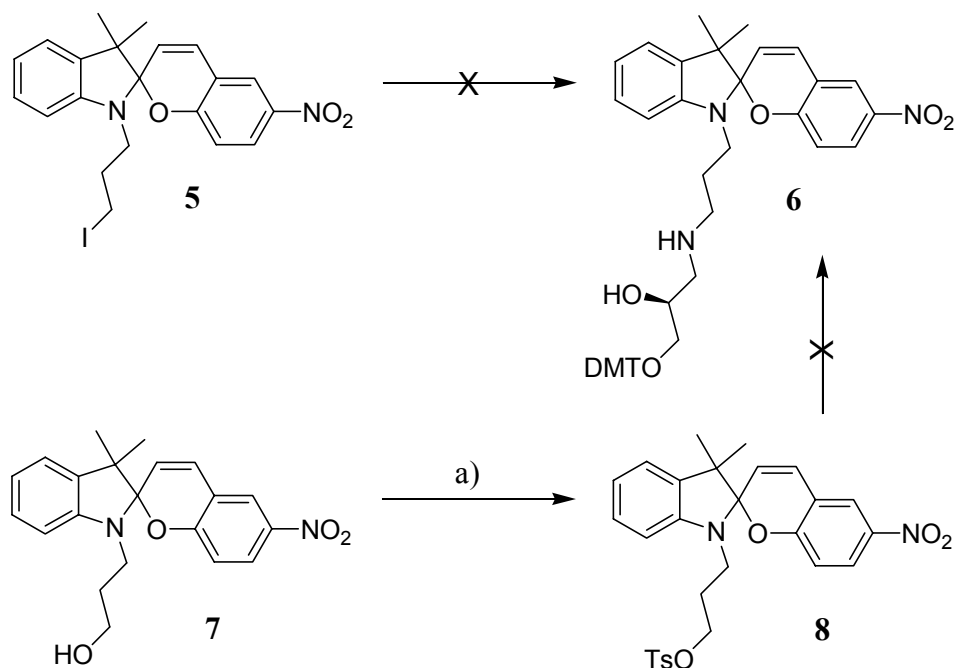


Scheme 2.1. Synthesis of acyclic linker **4**. Reagents and conditions: a) Methyltrifluoroacetate (10.0 eq.), r.t., 24 h, 90 %; b) DMTCl (1.0 eq.), NEt₃, pyridine, r.t., 60 h, 93 %; c) aq. NH₃ (94 eq.), MeOH, THF, r.t., 24 h, 89 %.

3.2.2. Synthesis of spiropyran iodide, tosylate and coupling to acyclic linker

Our initial attempt for the preparation of a DNA building block that consists of the spiropyrans unit and an acyclic sugar surrogate was to use a common strategy for the synthesis of **6** (Scheme 2.2). According to the literature, it should be possible to attach the spiropyrans **5** to the acyclic linker **4**, with addition of base in N,N-dimethylformamide.^[15b, 20]

In order to synthesize the spiropyrans-acyclic linker conjugate **6**, the iodide **5** was chosen. **5** was prepared in three steps with an overall yield of 66 %, according to the synthetic protocol that is discussed in Chapter 1. With the iodide as a good leaving group, a nucleophilic substitution with the primary amino-functionality of the acyclic linker system should occur. Thus, experiments under the described conditions were conducted,^[15b, 20] but none gave the desired product **6**.



Scheme 2.2. Coupling of spirobenzopyrans with acyclic linker compound. Reagents and conditions: a) p-TsCl (1.5 eq.), pyridine (1.5 eq.), THF, 0 °C to r.t., 21 h, 9 %.

As another route, the conversion of **7** into a tosylate was performed to facilitate a subsequent nucleophilic substitution reaction with the acyclic linker **4**. Therefore, the spirobenzopyran **7** was synthesized as described in Chapter 1. Then, the tosylation of spirobenzopyran compound was carried out with p-toluenesulfonyl chloride in the presence of pyridine in tetrahydrofuran. Although, a standard protocol was used for the tosylation, the desired compound **8** could only be isolated in low yield after column chromatography. Additionally, **8** could not be isolated by recrystallization on several attempts. Presumably the formation of pyridinium salt resulted in a concomitant loss of the desired tosylate.^[21] However, coupling procedures of the gained tosylate **8** with linker compound also resulted in no formation of the desired product **6**.

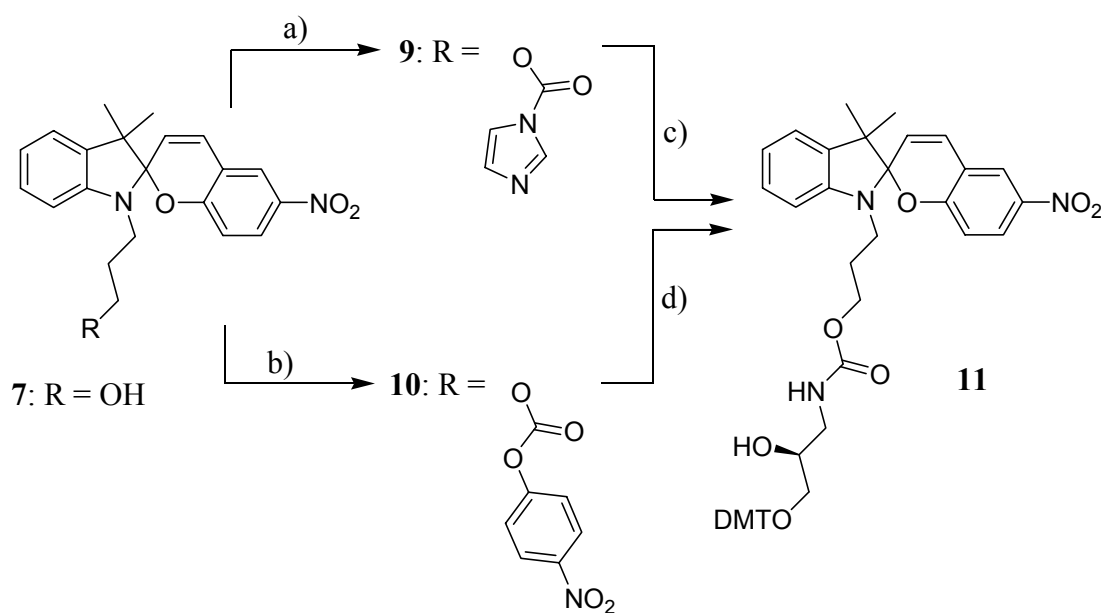
After all, the isolable quantities of tosylated spirobenzopyran **8** were not ample in the long run, and establishing steady reaction conditions to form the desired product **6** appeared very protracted. Thus, we decided to tackle a new route, where an elongation of the linkage between the spirobenzopyran and the acyclic linker was accepted. For practical reasons we designed our new approach particularly with regard to avoid mandatory tedious protection group chemistry that is associated with the acyclic linker.^[15b]

3.2.3. Synthesis of activated spirobenzopyrans and conjugation with acyclic linker

As previous attempts failed to synthesize the spiropyran-acyclic-linker conjugate **6** starting from iodo-alkylated spirobenzopyran **5** or the tosylated compound **8** via nucleophilic

substitution chemistry to the primary amino group of the acyclic linker **4**, it was important to embark on a new strategy.

For this reason, to activate the hydroxyl functions of **7** for nucleophilic attack of the amino functionality of **4**, a N-acylimidazole **9** and a mixed anhydride **10** were prepared (Scheme 2.3). These active intermediates liberate imidazole or p-nitrophenol, respectively, upon reaction with the amino-group, but not the carbonyl group.^[22] Accordingly, a one-carbon spacer is introduced and a stable N-alkyl carbamate (urethane) linkage is formed.



Scheme 2.3. Synthesis of activated spirobenzopyrans **9** together with **10** and coupling to the acyclic linker. Reagents and conditions: a) CDI (3.7 eq.), dioxane, r.t., 64 h, 51 %; b) 4-NPC (3.0 eq.), DIPEA, CH₂Cl₂, 0 °C, 3 h, 93 %; c) **4** (1.5 eq.), 4-DMAP (1.0 eq.), CH₂Cl₂, DMF, r.t., 67 h, 46 %; d) **4** (2.0 eq.), DIPEA (6.4 eq.), DMF, 0 °C, 5 h, 97 %.

The N-acylimidazole **9** was synthesized by reacting **7** with excess of 1,1'-carbonyldiimidazole (CDI). The reaction was carried out in dry dioxane at room temperature and **9** was obtained in 51 % yield as a yellow solid. The formed imidazolyl carbamate could then be coupled to the acyclic linker with its primary amino functionality in later reactions.

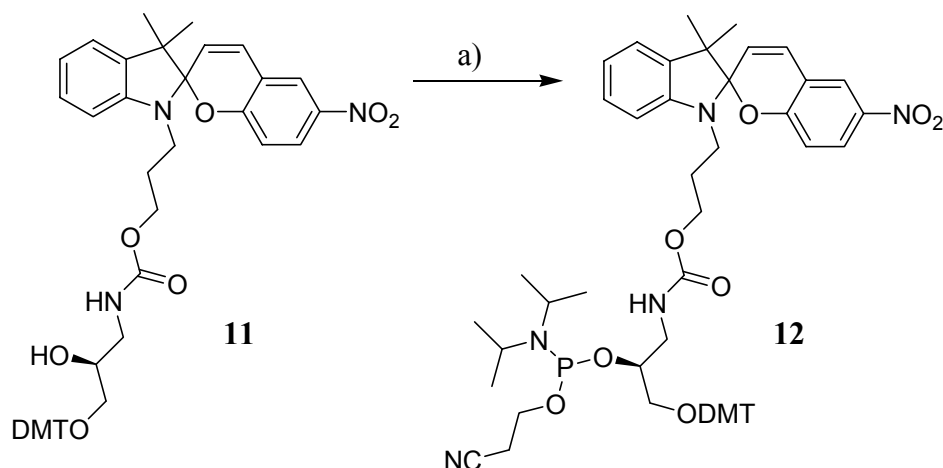
The mixed anhydride **10** was synthesized by reaction of **7** with 4-nitrophenylchloroformate (4-NPC), a popular reagent for the activation of alcohols towards the formation of carbamates and carbonates.^[23] According to assimilable procedures reported in the literature,^[24] an excess of 4-NPC was reacted with spirobenzopyran **7** at 0 °C in dry dichloromethane and addition of diisopropylethylamine for 3 hours. The resulting 4-NP carbonate was purified by flash chromatography and afforded **10** in 93 % yield.

Interestingly, equimolar amounts of activation agents and **7** did not show complete conversion to **9** or **10**, respectively. Both activation reactions worked smoothly with an excess of the activating agent. Accordingly, the in situ substitution of the 4-nitrophenyl moiety by addition of the amine was not possible at this step due to the excess of activation agent. Remarkably, the CDI-activated spirobenzopyran compound **9** appeared to be prone to hydrolysis, and decomposition was observed during chromatography where the original spirobenzopyran compound **7** reformed, explaining the lower yield (51 %). The observation of decomposition of CDI-activated labels has also been reported in the literature.^[22] However, the mixed anhydride compound **10** was stable during chromatography and did not show decomposition, and thus it was obtained in excellent yield (93 %).

Although, the conjugation reaction of **9** with the acyclic linker **4** was carried out in dry solvents to prevent hydrolysis, TLC displayed slow reaction and gave **11** in 46 % yield. On the other hand, the reaction of **10** with the acyclic linker **4** showed complete conversion within 5 hours and **11** was obtained in 97 % yield. Interestingly, in the meantime the preparation of CDI- or 4-NP-activated molecules has been adopted routinely by other members of our group and the strategy was used for successful conjugation reactions of different chromophores with the acyclic linker.^[25]

3.2.4. Synthesis of spirobenzopyran phosphoramidite and DNA

The final stage in the synthesis of the spirobenzopyran modified acyclic linker derivative is accomplished by addition of the phosphoramidite group. The appropriate cyanoethylphosphoramidite **12** was prepared by an optimized reaction of **11** with excess of commercially available 2-cyanoethyl-N,N-diisopropylchlorophosphoramidite **21** in dichloromethane with triethylamine at room temperature for 11 hours. The progress of the reaction was monitored with TLC, indicating complete conversion into the desired product **12**. However, it was important to purify the compound otherwise the incorporation during DNA synthesis functioned poorly. Thus, the phosphoramidite was carefully purified by flash chromatography on silica gel using hexanes-ethylacetate as an eluent that contains triethylamine as base to prevent cleavage of the trityl group. After chromatography both methods, lyophilization of **12** from benzene and solidification by precipitation, gave **12** in consistent and excellent yield.



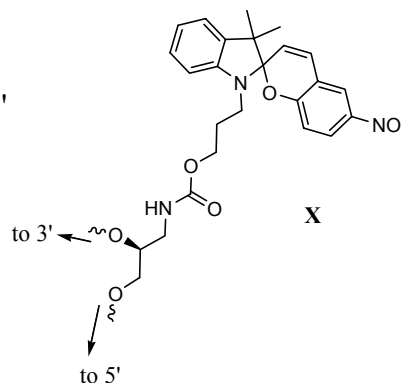
Scheme 2.4. Phosphitylation of spirobenzopyran acyclic linker. Reagents and conditions: a) **21** (2.0 eq.), CH₂Cl₂, NEt₃, r.t., 11 h, 95 %.

Following, the detritylated oligonucleotides **DNA 1a-1c** were synthesized automatically on the 1 μ mol scale using building block **12** in acetonitrile and a coupling time of 6.3 minutes. With respect to the expected chemical lability of the spirobenzopyran, the DNA was deprotected under very mild conditions at room temperature for 18 hours.

DNA 1a: 5'-GCA-GTC-TTX-TTC-ACT-GA-3'

DNA 1b: 5'-GCA-GTC-TAX-ATC-ACT-GA-3'

DNA 1c: 5'-GCA-GTC-TCX-CTC-ACT-GA-3'



The crude products were analyzed by HPLC, and, in fact, both isomers (spirobenzopyran and merocyanine) of the modified oligonucleotides were detectable by HPLC and could be identified by ESI mass spectrometry. However, the oligonucleotides were prone to decompose especially during the basic conditions for deprotection and cleavage. Due to the latter observation in combination with the low yield we decided to apply an alternative synthetic approach which is a so-called postsynthetic methodology. Alternatively, the use of different deprotection and cleavage systems might be considered for further experiments when the building block **12** is used for incorporation into DNA. E.g., ultrafast deprotection

and cleavage with AMA can be used as a reasonable alternative to ammonium hydroxide, but requires the substitution of Bz-dC with Ac-dC during DNA synthesis.^[26]

3.2.5. Synthesis of click dU and DNA

If labels or probes in DNA, like the spirobenzopyran modification or viologens,^[27] are chemically incompatible with the conditions under which DNA synthesis, deprotection or cleavage occur, postsynthetic modifications of presynthesized oligonucleotides can overcome these limitations and give access to the desired modification.^[28] Then, bioorthogonal ligation reactions are required where both, the functional group of the oligonucleotides and the functional group of the modifier should not be present in typical biomolecules and should react selectively with each other.^[29] The CuAAC click reaction matches the requirements since neither terminal alkyne nor azide functional groups are generally present in natural systems.^[30] Therefore, it has become the most important bioorthogonal labeling strategy and is introduced and discussed in Chapter 2.

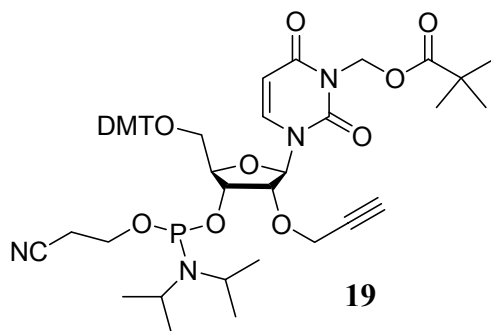
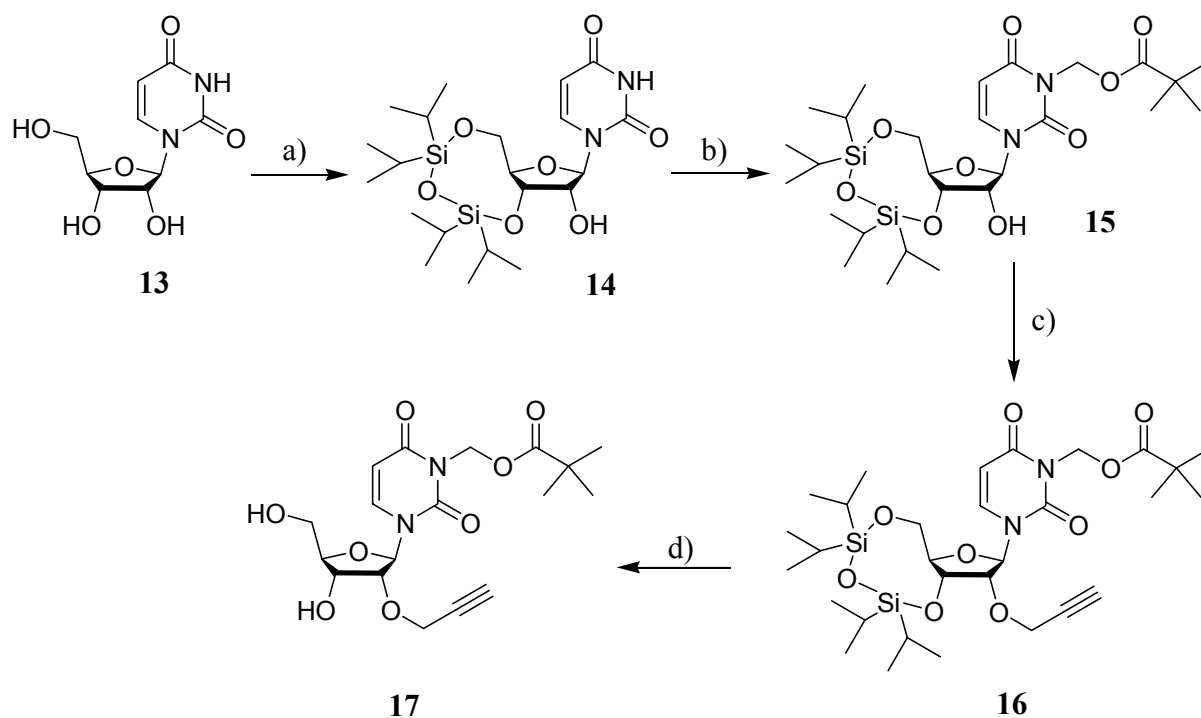


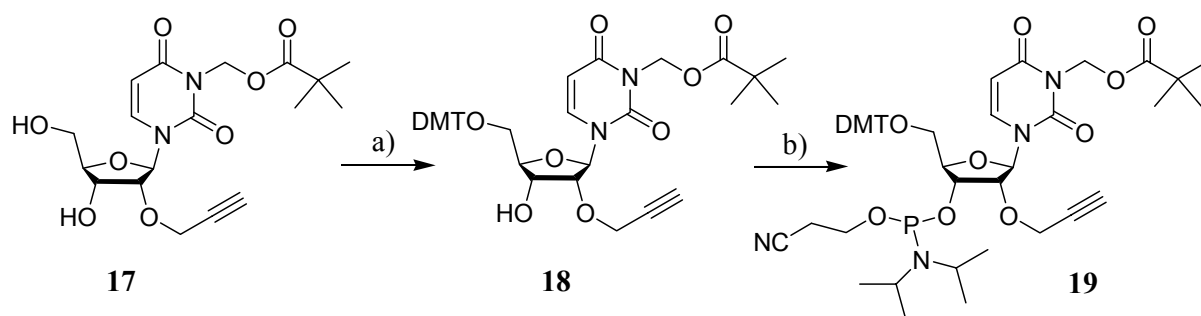
Figure 2.1. DNA building block with terminal alkyne functionality

As alkyne component for the click reaction we chose a 2'-O-propargyl-modified uridine **19** as phosphoramidite building block.^[31] Starting with commercially available uridine **13**, the 5'- and 3'-OH positions were first simultaneously protected with the bifunctional tetraisopropylidisiloxane (TIPDS) protection group ("Markiewicz group") under dry reaction conditions to afford **14** in 80 % yield. In the next step, the base-labile pivaloyloxymethyl (POM) protection group was introduced at the *N*³-position of the uracil base with TBAHS as a phase-transfer catalyst to yield **15** as glistening foam in 70 % yield. **15** was then reacted with 30 % excess of propargyl bromide and BEMP to give the 2'-O-propargyl-modified protected uridine **16**. Desilylation of the Markiewicz 3',5'-O-silyl protection group was carried out using NEt₃·3HF in THF and gave the desired nucleoside product **17** in 69 % yield.



Scheme 2.5. Synthesis of *N*³-POM-2'-alkynyl nucleoside **17**. Reagents and conditions: a) PG (1.1 eq.), pyridine, r.t., 6 h, 80 %; b) pivaloyloxymethyl chloride (10.0 eq.), TBAHS (25.1 mol-%), aq. Na₂CO₃ (10.2 eq.), CH₂Cl₂, r.t., 48 h, 70 %; c) Propargylbromide (1.3 eq.), BEMP (1.3 eq.), MeCN, 0 °C to r.t., 2 h, 30 %; d) NEt₃·3HF (4.0 eq.), THF, r.t., 16 h, 69 %.

To obtain the building block for DNA solid-phase synthesis, the 5'-OH group of **17** was protected with the acid-labile DMT protection group under addition of triethylamine to afford **18** in 92 % yield. Finally, the dimethoxytritylated compound was converted into the 2'-O-propargyluridine building block **19** by phosphorylation with 2-cyanoethyl-N,N-diisopropylchlorophosphoramidite **21** and triethylamine in dichloromethane. Purification by flash chromatography on a short column and precipitation with hexane in ethyl acetate gave **19** as colorless crystals in 93 % yield.



Scheme 2.6. Synthesis of 2'-alkynyl-phosphoramidite building block **19**. Reagents and conditions: a) DMTCI (1.2 eq.), NEt₃ (3.2 eq.), pyridine, r.t., 16 h, 92 %; b) **21** (3.2 eq.), NEt₃ (12.5 eq.), CH₂Cl₂, r.t., 5 h, 93 %.

In order to synthesize the spirobenzopyran-conjugated DNA we performed CuAAC with a spirobenzopyran azide and presynthesized DNA bearing terminal alkyne groups. The spirobenzopyran **20** was chosen as the azide component for the following click reactions, and it was synthesized in four steps with an overall yield of 55 %, according to the synthetic protocol that is discussed in detail in Chapter 1.

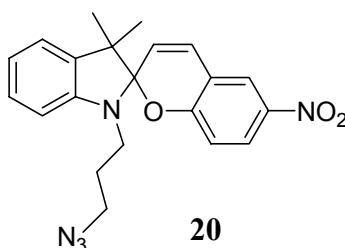
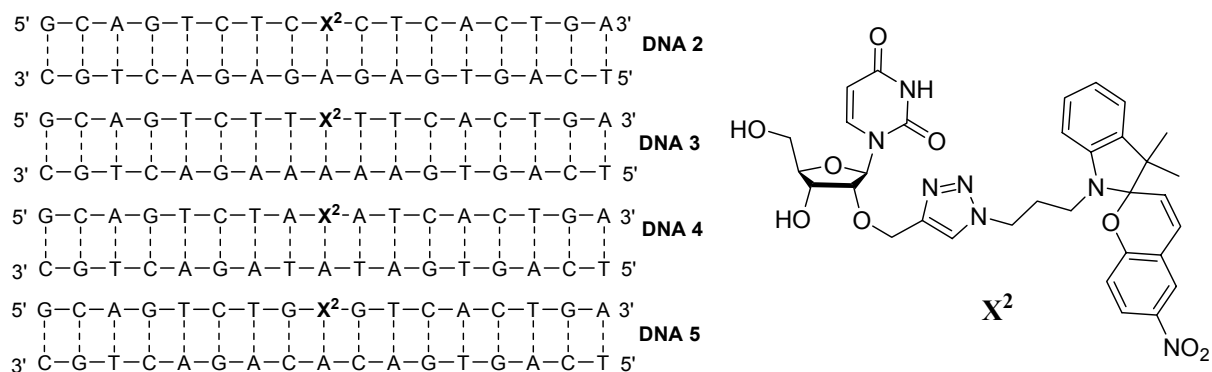


Figure 2.2. Spirobenzopyran azide for DNA click reactions

For the preparation of the oligonucleotides, we increased the concentration of phosphoramidite **19** to 0.1 M and the coupling time was extended to 6.3 minutes. After preparation, the trityl-off oligonucleotides were cleaved from the solid phase and deprotected by treatment with conc. NH₄OH at room temperature for 24 hours. For the click ligations the cleaved oligonucleotides were treated with spirobenzopyran azide **20** in the presence of Cu(I), TBTA and sodium ascorbate in a solvent mixture (DMSO/^tBuOH/H₂O) at room temperature for 22 hours. At last, the oligonucleotides were desalted and purified.^[17b, 17e] The modified oligonucleotides could be identified by ESI mass spectrometry and finally hybridized with 1.2 eq. of the corresponding unmodified counterstrands to the spirobenzopyran-modified DNA duplexes **DNA 2-5**.



The melting temperatures (T_m) of the spirobenzopyran-modified duplexes were measured (Table 2.1). As expected for a twisted structure like the spirobenzopyrans the duplexes show a significant destabilization when compared with the melting temperatures (T_m') of their corresponding unmodified duplex ($X^2 = T$).

Duplex	T_m (°C)	T_m' (°C)	ΔT_m
DNA 2	47.9	66.0	-18.1
DNA 3	50.0	62.5 ^[17e]	-12.5
DNA 4	48.0	61.0	-13.0
DNA 5	47.8	68.0 ^[17e]	-20.2

Table 2.1. Melting temperatures (T_m) of duplexes **DNA 2** - **DNA 5** in comparison to the unmodified duplexes. Duplex (2.5 μ M) in Na-P_i buffer (10 mM), NaCl (250 mM)

The UV/Vis absorption spectra of the duplexes (Figure 2.3) clearly revealed the presence of the spirobenzopyran modification by the broad absorption band between 300 nm and 420 nm. **DNA5** shows the strongest extinction which may be due to stacking with guanine. The very weak absorption of **DNA 2** - **DNA 5** between 500 and 600 nm seems to represent a small amount of the merocyanine form which may result of a preset equilibrium between the spirobenzopyran and open merocyanine form during the synthetic and preparative procedures.

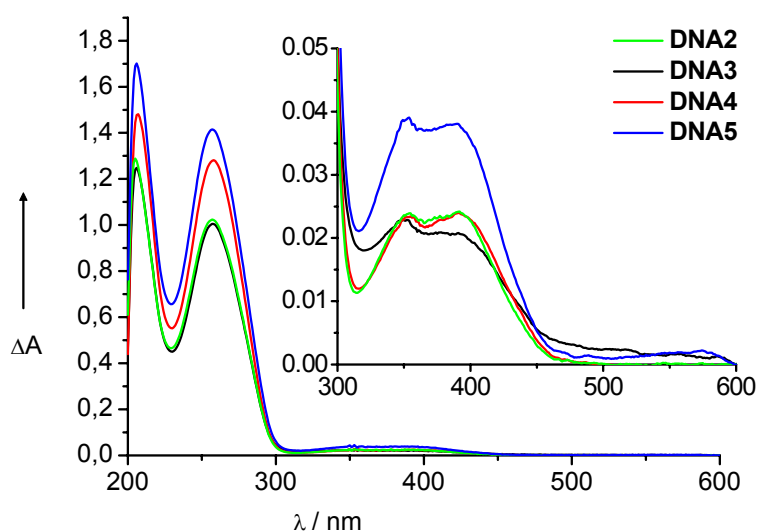


Figure 2.3. UV/Vis absorption spectra of **DNA 2 - DNA 5** (2.5 μM) in Na-P_i buffer (10 mM), NaCl (250 mM)

It was unexpected, however, that irradiation with UV light at $\lambda = 312$ and 366 nm did not initiate the ring opening of the spirobenzopyran-modified DNA duplexes to the corresponding merocyanine-modified ones. It is important to point out that the irradiation conditions were sufficient to switch the spirobenzopyran derivatives without DNA. Obviously, the presence of DNA in the neighborhood of the spirobenzopyran moiety inhibits the photoopening process to the merocyanine. According to the literature,^[32] the presence of the nitro group at the 6-position of the benzopyran moiety should shift the equilibrium towards the open merocyanine form by enhancing the quantum yield of intersystem crossing and thereby favoring a triplet pathway for photocoloration. On the other hand, it was found that polar solvents decrease the quantum yield for the triplet pathway of photocoloration significantly.

Under the assumption that the polarity of the DNA base stack is not extremely high, it seems to be very likely that the DNA bases quench the triplet state of the spirobenzopyran by energy or electron transfer processes thereby inhibiting the photoswitching process. Further work by time-resolved laser spectroscopy could be used to elucidate these processes. Nevertheless, it evinced that the spirobenzopyran chromophore cannot be simply applied as such for switching DNA hybridization but must be further developed and tuned by chromophore substituents to achieve this goal.

3.3. Conclusion

In conclusion, the photochromic spirobenzopyran was incorporated as an internal modification into oligonucleotides by two different synthetic strategies: For the first route the spirobenzopyran phosphoramidite was synthesized as a DNA building block phosphoramidite from scratch. Therefore, an acyclic linker system was prepared and the CDI- or 4-NP-activated spirobenzopyran was linked as a N-alkyl carbamate. The presented prolongation of the acyclic linker has turned out to be a very expedient synthetic method. Decomposition during deprotection of the modified oligonucleotides led to a second route where the postsynthetic click-type ligation was successfully applied. There, melting temperature measurements of the spirobenzopyran-modified duplexes displayed significant destabilization compared to unmodified DNA. However, photoinduced ring opening of the chromophore could not be achieved in duplex DNA. Further tuning of the chromophore substituents and time-resolved spectroscopy or testing of other photoswitchable compounds could help to find molecules that might be better suited for photoswitchable DNA hybridization. Alternative deprotection and cleavage conditions (e.g., AMA) as well as purification of the synthesized oligonucleotides by gel electrophoresis can develop investigations of spirobenzopyran-modified DNA further.

3.4. Experimental Section

General

Assignable details on reagents, solvents, reaction processing, chromatography, NMR and MS are specified in the general section of the experimental part in Chapter 1. Compounds **5**, **7** and **20** were prepared according to the synthetic procedures reported in the experimental section in Chapter 1. 2-cyanoethyl-N,N-diisopropylchlorophosphoramidite **21** was obtained from commercial suppliers. Purified water with a resistivity $\geq 18 \text{ M}\Omega \text{ cm}^{-1}$ was used for preparation of buffer solvents, a MARTIN CHRIST Alpha 2-4 freeze dryer was used for lyophilization and oligonucleotides were desalted with use of prepacked illustra NAP-5 columns from GE Healthcare and NH_4OAc buffer (5 mM). Unmodified oligonucleotides were purchased from Metabion.

RP-HPLC

RP-HPLC was performed with use of a Shimadzu instrument (Autosampler SIL-10AD, Pump module LC-10AT, Control unit SCL-10A, Multidiodearray detector SPD-M10A). For oligonucleotide analysis, a Supelcosil LC-318 column (250 mm x 4.6 mm), for semi-preparative purifications a Supelcosil LC-318 column (250 mm x 10 mm, ID, 5 μ m) were used. Solvents used: A) NH_4OAc buffer (50 mM), pH 6.8; B) acetonitrile. Analytical separations were performed with a flow rate of 1.0 mL/min, semi-preparative separations were performed with a flow rate of 2.5 mL/min. Unless mentioned otherwise, oligonucleotides were detected by their absorption at 260 nm.

DNA solid support synthesis

Oligonucleotides were prepared on an Expedite 8909 Synthesizer from Applied Biosystems (ABI) using standard phosphoramidite chemistry on a 1 μ mol scale. Reagents and controlled pore glass (CPG) were purchased from ABI and Glen Research. The concentration of the modified DNA building blocks was increased to 0.1 M (in MeCN) and the coupling time was extended to 6.3 min. Further details are given in the appendix. **DNA 1a-c** were cleaved from the resin and deprotected by treatment with concentrated NH_4OH at r.t. for 18 h.

Synthesis of oligonucleotides: Off bead coupling protocol and purification

The 2'-modified uridine **19** was introduced into DNA by using standard coupling conditions, extended coupling time (6.3 min) and increased concentration of 0.1 M (in MeCN). The oligonucleotides were cleaved from the resin and deprotected by treatment with concentrated NH_4OH solution at r.t. for 24 h. Compound **20** (1.5 mL, 10 mM), CuI (223.6 μ L, 100 mM), TBTA (447 μ L, 100 mM), each in DMSO/*t*BuOH (3:1), and sodium ascorbate (125 μ L, 400 mM) in H_2O were added to the oligonucleotide (1 μ mol). The vial was vortexed, shaken for 22 h at r.t., and then evaporated to dryness using a SpeedVac. NaOAc (100 μ L, 0.15 mM) was added, and the mixture was stored for 1 h at r.t. After EtOH precipitation, the oligonucleotides were dissolved in H_2O (500 μ L), desalted, dried and purified by RP-HPLC using the following conditions: A) NH_4OAc buffer (50 mM), pH 6.8; B) acetonitrile; gradient 0-15 % B over 45 min, flow rate 2.5 mL/min and lyophilized. The oligonucleotides were quantified in 10 mM sodium phosphate buffer by their absorbance at 260 nm using $\epsilon_{260\text{nm}} = 11000 \text{ M}^{-1} \text{ cm}^{-1}$ for X^2 , $\epsilon_{260\text{nm}} = 13800 \text{ M}^{-1} \text{ cm}^{-1}$ for A, $\epsilon_{260\text{nm}} = 10500 \text{ M}^{-1} \text{ cm}^{-1}$ for G, $\epsilon_{260\text{nm}} = 8000 \text{ M}^{-1} \text{ cm}^{-1}$ for T, $\epsilon_{260\text{nm}} = 6500 \text{ M}^{-1} \text{ cm}^{-1}$ for C. $\epsilon_{260\text{nm}}$ of the respective DNA strand: the

number of individual bases was multiplied with their respective extinction coefficients and added together.

UV/Vis spectroscopy and melting temperature measurements of DNA

Unless otherwise specified, spectroscopic measurements were performed at 20 °C and quartz glass cuvettes (Starna, 10 mm) were used. UV/Vis spectra were recorded with a Cary BIO 100 UV/Vis/NIR spectrometer (Varian) with temperature-controlled 6x6 cuvette holder.

Hybridization and melting temperatures

Duplexes were formed by heating of the modified oligonucleotides (2.5 µM) in 10 mM sodium phosphate buffer (pH 7) and 250 mM NaCl in the presence of 1.2 eq. unmodified complementary strand to 90 °C (10 min), followed by slow cooling to r.t. Melting temperatures of the duplexes (20-90 °C, 0.7 °C/min, step width 0.5 °C) were recorded on a Varian Cary 100 spectrometer equipped with a temperature-controlled 6×6 cell changer unit.

Light sources

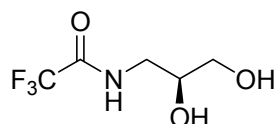
For irradiation experiments a UV hand-held lamp (Herolab, 6 W, $\lambda = 312$ nm), a UV hand-held lamp (Faust, 2 x 4 W, $\lambda = 366$ nm), a mercury-vapor lamp (500 W) with interference filter ($\lambda = 367.55$ nm, HW = 12.9 nm) and a Luxeon III Star high-power LED ($\lambda = 590$ nm / amber) were used.

Table 4.1. ESI-MS of modified oligonucleotide single strands (ss).

ss DNA	Sequence	calcd.	found
DNA 1a	5'-GCA-GTC-TTX-TTC-ACT-GA-3'	5390.0	1346.8 [M-4H ⁺] ⁴⁻ , 1796.1 [M-3H ⁺] ³⁻
DNA 1b	5'-GCA-GTC-TAX-ATC-ACT-GA-3'	5408.0	1351.3 [M-4H ⁺] ⁴⁻ , 1802.1 [M-3H ⁺] ³⁻
DNA 1c	5'-GCA-GTC-TCX-CTC-ACT-GA-3'	5360.0	1339.4 [M-4H ⁺] ⁴⁻ , 1786.2 [M-3H ⁺] ³⁻
DNA 2	5'-GCA-GTC-TCX ² -CTC-ACT-GA-3'	5550.0	1387.4 [M-4H ⁺] ⁴⁻ , 1850.5 [M-3H ⁺] ³⁻
DNA 3	5'-GCA-GTC-TTX ² -TTC-ACT-GA-3'	5580.0	1395.0 [M-4H ⁺] ⁴⁻ , 1860.1 [M-3H ⁺] ³⁻
DNA 4	5'-GCA-GTC-TAX ² -ATC-ACT-GA-3'	5598.1	1399.3 [M-4H ⁺] ⁴⁻ ,

			1866.5 [M-3H ⁺] ³⁻
DNA 5	5'-GCA-GTC-TGX ² -GTC-ACT-GA-3'	5630.1	1407.5 [M-4H ⁺] ⁴⁻ , 1876.7 [M-3H ⁺] ³⁻

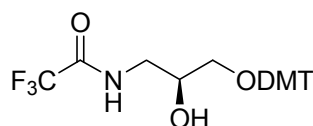
2,2,2-trifluoro-N-((S)-2,3-dihydroxypropyl)acetamide



A mixture of (S)-3-amino-1,2-propanediol (1.00 g, 10.98 mmol) and methyl trifluoroacetate (11.0 mL, 109.36 mmol) was stirred at room temperature for 24 hours. Following, the reaction mixture is concentrated in vacuo and residual solvent was coevaporated using toluene. 2,2,2-trifluoro-N-((S)-2,3-dihydroxypropyl)acetamide was obtained as gooey pale yellow oil (1.845 g, 90 %).

The analytical data was consistent with the literature.^[15b, 16]

N-((S)-3-(bis(4-methoxyphenyl)(phenyl)methoxy)-2-hydroxypropyl)-2,2,2-trifluoroacetamide

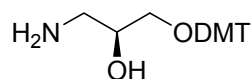


A flask was charged with 2,2,2-trifluoro-N-((S)-2,3-dihydroxypropyl)acetamide (2.264 g, 12.10 mmol), dry pyridine (36 mL) and 4,4'-dimethoxytriphenylmethyl chloride (4.093 g, 12.08 mmol). The solution was degassed, dry NEt₃ (2.0 mL) was added under nitrogen atmosphere and the reaction mixture was stirred in the dark at room temperature for 60 hours. Following, the solvents were removed under reduced pressure, EtOAc (150 mL) and an aqueous saturated NaHCO₃ solution (150 mL) were added. The aqueous layer was extracted again with EtOAc, the organic layers were pooled and dried over anhydrous Na₂SO₄. The solvent was removed under reduced pressure and the crude product was purified by gradient

flash chromatography on silica gel ($\text{CH}_2\text{Cl}_2 + 1\% \text{NEt}_3$ to $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 50:1 + 1% NEt_3) to afford the title compound as glistening pale yellow foam (5.515 g, 93 %). $R_f = 0.10$ ($\text{CH}_2\text{Cl}_2 + 1\% \text{NEt}_3$)

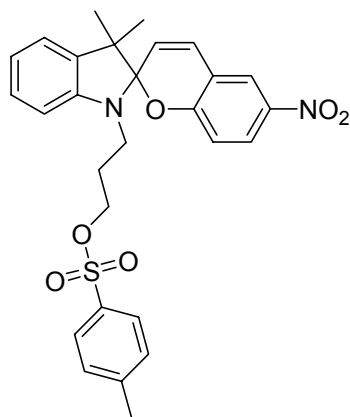
^1H NMR (300 MHz, CDCl_3): $\delta = 7.44\text{--}7.37$ (m, 2 H, arom.), 7.32–7.26 (m, 7 H, arom.), 6.86–6.82 (m, 4 H, arom.), 3.98–3.97 (m, 1 H), 3.79 (s, 6 H, 2 x OCH_3), 3.67–3.56 (m, 1 H), 3.36–3.21 (m, 2 H), 3.18–3.10 (m, 1H) - ^{13}C NMR (75 MHz, CDCl_3): $\delta = 158.7, 157.2, 149.7, 136.1, 130.0, 129.2, 128.0, 127.1, 123.8, 113.3$ (+, CH), 86.6, 68.9 (+, CH), 64.8 (-, CH_2), 55.2 (+, CH_3 , 2 x OCH_3), 46.1 (-, CH_2)

(S)-1-amino-3-(bis(4-methoxyphenyl)(phenyl)methoxy)propan-2-ol



A flask was charged with N-((S)-3-(bis(4-methoxyphenyl)(phenyl)methoxy)-2-hydroxypropyl)-2,2,2-trifluoroacetamide (3.525 g, 7.21 mmol), MeOH (50 mL), THF (23 mL) and capped with rubber septum. A solution of ammonia (50 mL, 25 wt %, in water) was added and the reaction mixture was stirred at room temperature in the dark for 24 hours. Following, the volatile solvents were evaporated under reduced pressure and afforded the mucous crude product. CH_2Cl_2 (60 mL) and water (40 mL) were added, the aqueous layer was extracted with CH_2Cl_2 (2 x 50 mL), the organic layers were pooled, and concentrated to a total volume of 50 mL. The organic layer was washed with brine (2 x 50 mL) and dried over anhydrous Na_2SO_4 . The solvent was removed under reduced pressure to yield (S)-1-amino-3-(bis(4-methoxyphenyl)(phenyl)methoxy)propan-2-ol as colorless fuzzy foam (2.530 g, 89 %).

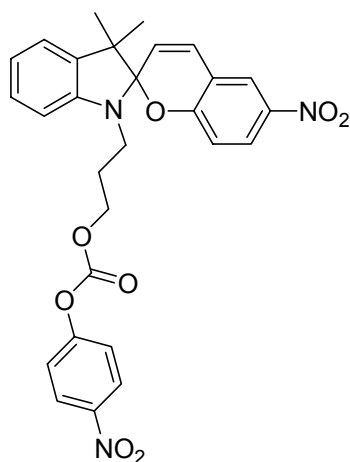
The analytical data was consistent with the literature.^[15b, 16]

3',3'-Dimethyl-6-nitro-Spiro[2H-1-benzopyran-2,2'-[2H]indole]-1'(3'H)-3-propyl-4-methylbenzenesulfonate

3',3'-Dimethyl-6-nitro-Spiro[2H-1-benzopyran-2,2'-[2H]indole]-1'(3'H)-propanol (532 mg, 1.45 mmol) was dissolved in dry THF (8.0 mL) and dry pyridine (230 μ L, 2.17 mmol) was added. The mixture was cooled to 0 $^{\circ}$ C and p-toluenesulfonyl chloride (414 mg, 2.17 mmol) was added in portions over 1 hour. The mixture was allowed to slowly warm up to ambient temperature and after 20 hours the solvents were evaporated under reduced pressure to give the crude product as yellow-brown oil. Purification by gradient flash chromatography on silica gel (Hexane/EtOAc 10:2 to 10:4) afforded 3',3'-Dimethyl-6-nitro-Spiro[2H-1-benzopyran-2,2'-[2H]indole]-1'(3'H)-3-propyl-4-methylbenzenesulfonate as pale pink solid (68 mg, 9 %). R_f = 0.37 (Hexane/EtOAc 10:2)

^{13}C NMR (150 MHz, CDCl_3): δ = 150.8, 147.7, 146.4, 146.3, 138.7, 133.4, 132.4, 132.2, 130.3, 128.3, 127.5, 126.6, 123.9, 123.6, 122.4, 122.0, 119.6, 108.8, 100.6, 62.7, 49.7, 40.2, 27.2, 22.0, 21.7, 18.5 - MS (ESI): m/z (%): 521.2 (100) $[\text{MH}^+]$

4-Nitrophenyl-2-(3',3'-dimethyl-6-nitro-3'H-spiro[chromene-2,2'-indole]-1'-yl)propylcarbonate

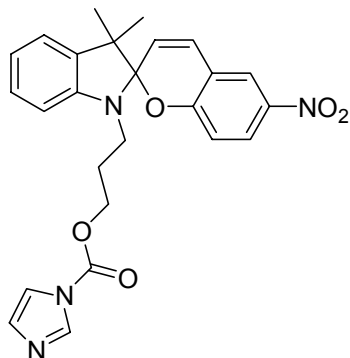


A solution of 3',3'-Dimethyl-6-nitro-Spiro[2H-1-benzopyran-2,2'-[2H]indole]-1'(3'H)-propanol (1.060 g, 2.89 mmol) in dry CH_2Cl_2 (22 mL) was cooled to 0 °C and dry DIPEA (2.2 mL, 12.94 mmol) was added under argon atmosphere. 4-Nitrophenylchloroformate (1.765 g, 8.76 mmol) was dissolved in dry dichloromethane (16.5 mL) and added in small portions over 3 hours. The reaction was slowly warmed up to ambient temperature, the solvent was evaporated and the crude product was dried over night under vacuum. The crude material was purified by flash chromatography on silica gel (Petrolether/EtOAc 2:1). Evaporation of the solvent afforded a pale pink solid that was repeatedly purified by flash chromatography on silica gel (Toluene). 4-Nitrophenyl-2-(3',3'-dimethyl-6-nitro-3'H-spiro[chromene-2,2'-indole]-1'-yl)propylcarbonate was triturated with Et_2O and obtained as a pale yellow solid (1.436 g, 93 %).

^1H NMR (400 MHz, CDCl_3): δ = 8.28 (d, 2 H, J = 9.3 Hz, H-Ar), 8.05-7.98 (m, 2 H, H-Ar), 7.34 (d, 2 H, J = 9.3 Hz, H-Ar), 7.20 (dt, 1 H, J = 1.3, 7.7 Hz, H-Ar), 7.11 (dd, 1 H, J = 0.9, 7.3 Hz, H-Ar), 6.91 (t, 2 H, J = 8.6 Hz, H-Ar), 6.77 (d, 1 H, J = 8.8 Hz, H-Ar), 6.61 (d, 1 H, J = 7.7 Hz, H-Ar), 5.88 (d, 1 H, J = 10.3 Hz, H-Ar), 4.34 (dt, 2 H, J = 1.7, 5.9 Hz, CH_2 -propyl), 3.44-3.28 (m, 2 H, CH_2 -propyl), 2.19-1.98 (m, 2 H, CH_2 -propyl), 1.30 (s, 3 H, CH_3), 1.20 (s, 3 H, CH_3) - ^{13}C NMR (100 MHz, CD_2Cl_2): δ = 162.0 ($\text{C}_{\text{quat.}}$), 159.9 ($\text{C}_{\text{quat.}}$), 156.0 ($\text{C}_{\text{quat.}}$), 152.9 ($\text{C}_{\text{quat.}}$), 147.3 ($\text{C}_{\text{quat.}}$), 145.9 ($\text{C}_{\text{quat.}}$), 141.5 ($\text{C}_{\text{quat.}}$), 136.6 ($\text{C}_{\text{quat.}}$), 128.9 (+, CH), 128.2 (+, CH), 126.5 (+, CH), 126.3 (+, CH), 125.7 (+, CH), 123.2 (+, CH), 122.3 (+, CH), 122.3 (+, CH), 122.0 (+, CH), 120.2 (+, CH), 119.1 ($\text{C}_{\text{quat.}}$), 116.1 (+, CH), 115.9 (+, CH), 107.3 ($\text{C}_{\text{quat.}}$), 107.0 (+, CH), 67.6 (-, CH_2), 40.5 (-, CH_2), 28.3 (-, CH_2), 26.1 (+, CH_3), 20.0 (+,

CH₃) - IR (neat): ν [cm⁻¹] = 2970, 2359, 1740, 1217 - MS (ESI): m/z (%): 532.3 (100) [MH⁺]
 - HRMS (PI-EI) calcd. for C₂₈H₂₅N₃O₈ [M⁺]: 531.1642, found: 531.1639

2-(3',3'-dimethyl-6-nitro-3'H-spiro[chromene-2,2'-indole]-1'-yl)propyl-1H-imidazole-1-carboxylate

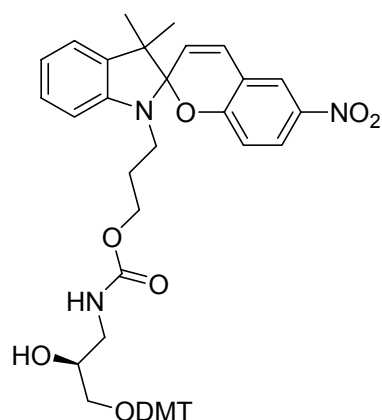


3',3'-Dimethyl-6-nitro-Spiro[2H-1-benzopyran-2,2'-[2H]indole]-1'(3'H)-propanol (366 mg, 1.00 mmol) was dissolved in dry dioxane (40 mL) and the purple solution was stirred for 1 hour at room temperature in the dark. Following, 1,1'-carbonyldiimidazole (172 mg, 1.06 mmol) was added and the mixture was stirred vigorously for 24 hours. Reaction control using TLC showed non-complete consumption of the starting material, 1,1'-carbonyldiimidazole (431 mg, 2.66 mmol) was additionally added. After 64 hours the solvent was evaporated, the crude product was dried and purified by gradient flash chromatography on silica gel (Hexane/EtOAc 2:1 to 1:1). 2-(3',3'-dimethyl-6-nitro-3'H-spiro[chromene-2,2'-indole]-1'-yl)propyl-1H-imidazole-1-carboxylate was triturated with Et₂O and obtained as yellow solid (235 mg, 51 %). R_f = 0.29 (Hexane/EtOAc 1:1)

¹H NMR (600 MHz, CDCl₃): δ = 8.07 (dd, 1 H, J = 0.9, 1.3 Hz, imidazol, N=CH-N), 8.00 (dd, 1 H, J = 2.7, 9.0 Hz, C7-H), 7.93 (dd, 1 H, J = 0.5, 2.7 Hz, C5-H), 7.33 (dd, 1 H, J = 1.3, 1.7 Hz, imidazol, CH=CH-N), 7.19 (ddd, 1 H, J = 1.3, 7.6, 7.7 Hz, C7'-H), 7.10 (ddd, 1 H, J = 0.4, 1.3, 7.7 Hz, C5'-H), 7.07 (dd, 1 H, J = 0.9, 1.7 Hz, imidazol, CH=CH-N), 6.90 (ddd, 1 H, J = 1.0, 7.3, 7.6 Hz, C6-H), 6.82 (d, 1 H, J = 10.3 Hz, C4-H), 6.74 (dd, 1 H, J = 0.5, 9.0 Hz, C8-H), 6.58 (ddd, 1 H, J = 0.5, 1.0, 7.7 Hz, C8'-H), 5.83 (d, 1 H, J = 10.3 Hz, C3-H), 4.51-4.45 (m, 2 H, O-CH₂-propyl), 3.45-3.36 (m, 1 H, -CH₂-CH₂-CH₂-), 3.34-3.28 (m, 1 H, -, -CH₂-CH₂-CH₂-), 2.25-2.16 (m, 1 H, N-CH₂-propyl), 2.14-2.07 (m, 1 H, N-CH₂-propyl), 1.30 (s, 3

H, CH₃), 1.20 (s, 3 H, CH₃) - ¹³C NMR (150 MHz, CDCl₃): δ = 159.2 (C_{quat.}, C6-NO₂), 148.5 (C_{quat.}, imidazol-N-(C=O)-O), 146.5 (C_{quat.}), 141.1 (C_{quat.}), 136.9 (imidazol, N=CH-N), 136.0 (C_{quat.}), 130.7 (imidazol, CH=CH-N), 128.3 (CH, C4-H), 127.8 (CH, C7'-H), 125.9 (CH, C7-H), 122.7 (CH, C5-H), 121.9 (CH, C5'-H), 121.4 (CH, C3-H), 120.0 (CH, C6'-H), 118.2 (C_{quat.}), 116.9 (imidazol, CH=CH-N), 115.5 (CH, C8-H), 106.5 (C_{quat.}), 106.5 (CH, C8'-H), 65.9 (O-CH₂-propyl), 52.6 (C_{quat.}), 40.1 (-CH₂-CH₂-CH₂-), 27.7 (N-CH₂-propyl), 25.9 (+, CH₃), 19.9 (+, CH₃) - IR (neat): ν [cm⁻¹] = 2970, 2365, 1744, 1366 - MS (ESI): m/z (%): 461.2 (100) [MH⁺] - HRMS (PI-EI) calcd. for C₂₅H₂₄N₄O₅ [M⁺]: 460.1747, found: 460.1746

[3',3'-Dimethyl-6-nitro-Spiro[2H-1-benzopyran-2,2'-[2H]indole]-1'(3'H)]propyl-(S)-3-(bis(4-methoxyphenyl)(phenyl)methoxy)-2-hydroxypropylcarbamate



Route 1:

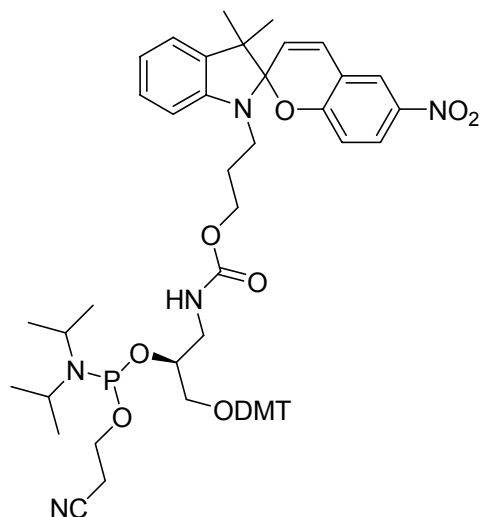
A solution of 4-Nitrophenyl-2-(3',3'-dimethyl-6-nitro-3'H-spiro[chromene-2,2'-indole]-19-yl)propylcarbonate (25 mg, 0.047 mmol) in dry DMF (5 mL) was cooled to 0 °C. Dry DIPEA (50 μL, 0.3025 mmol) was added under nitrogen and stirred for 5 minutes. (S)-1-Amino-3-[bis-(4-methoxy-phenyl)-phenylmethoxy]-propan-2-ol (37 mg, 0.0941 mmol) was added under nitrogen and the reaction mixtures was stirred at 0 °C over 5 hours. The reaction was slowly warmed to room temperature. The reaction mixture was concentrated in vacuo and purified by gradient flash chromatography on silica gel (Petrolether/EtOAc 2:1 + 0.1 % DIPEA to 1:1 + 0.1 % DIPEA). The title compound was obtained as a pale pink solid (36 mg, 97 %). R_f = 0.49 (Hexane/EtOAc 2:1 + 0.1 % DIPEA)

Route 2:

To a solution of 2-(3',3'-dimethyl-6-nitro-3'H-spiro[chromene-2,2'-indole]-1'-yl)propyl-1H-imidazole-1-carboxylate (132 mg, 0.287 mmol) in a 1:2 mixture of dry CH₂Cl₂/DMF (24 mL), (S)-1-amino-3-[bis-(4-methoxy-phenyl)-phenylmethoxy]-propan-2-ol (169 mg, 0.430 mmol) and 4-DMAP (36 mg, 0.295 mmol) were added under argon atmosphere. The reaction mixture was stirred at room temperature for 67 hours. Following, the reaction mixture was concentrated in vacuo and purified by preparative thin layer chromatography on silica gel (Hexane/EtOAc 2:1 + 0.1 % NEt₃) to afford the title compound as pink foam (104 mg, 46 %).

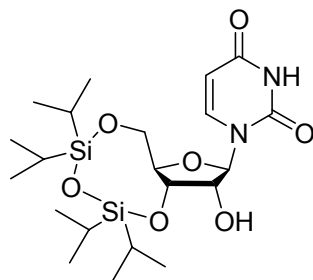
¹H NMR (400 MHz, CDCl₃): δ = 8.05-7.93 (m, 2 H), 7.41 (d, 2 H, J = 7.3 Hz), 7.33-7.27 (m, 6 H), 7.24-7.15 (m, 2 H), 7.09 (dd, 1 H, J = 0.8, 7.2 Hz), 6.90-6.81 (m, 6 H), 6.74 (d, 1 H, J = 8.9 Hz), 6.57 (d, 1 H, J = 7.7 Hz), 5.84 (d, 1 H, J = 10.4 Hz), 4.96 (s, 1 H, OH), 4.15-4.01 (m, 3 H), 3.90-3.83 (m, 1 H, CH-OH), 3.78 (s, 6 H, 2 x OCH₃), 3.39 (s, 1 H, NH), 3.32-3.25 (m, 1 H), 3.25-3.12 (m, 4 H), 2.01-1.92 (m, 1H), 1.90-1.83 (m, 1 H), 1.28 (s, 3 H), 1.18 (s, 3 H) - ¹³C NMR (100 MHz, CDCl₃): δ = 159.5, 158.6, 146.9, 144.5, 141.0, 136.0, 135.7, 130.0, 128.2, 128.0 (CH), 128.0, 127.8, 126.9, 125.9 (CH), 122.7 (CH), 121.7, 119.6 (CH), 118.5, 115.5 (CH), 113.2 (CH), 106.7 (CH), 106.7 (CH), 86.3, 70.3, 70.3, 65.0, 64.9, 62.5, 60.4, 55.2 (OCH₃), 52.6, 44.1, 40.3, 31.5, 28.2, 28.2, 25.9 (CH₃), 25.5, 21.0, 19.8 (CH₃), 14.2 - MS (ESI): m/z (%): 786.4 (100) [MH⁺] - HRMS (PI-MS) calcd. for C₄₆H₄₇N₃O₉ [M⁺]: 785.3312, found: 785.3325

[3',3'-Dimethyl-6-nitro-Spiro[2H-1-benzopyran-2,2'-[2H]indole]-1'(3'H)]propyl-(S)-3-(bis(4-methoxyphenyl)(phenyl)methoxy)-2-O-(2-cyanoethyl-N,N-diisopropylphosphoramidite)-propylcarbamate



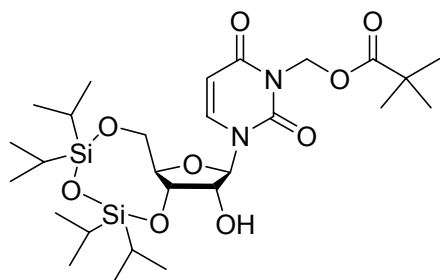
Compound (100 mg, 0.127 mmol) was dissolved in dry CH_2Cl_2 (5 mL). NEt_3 (250 μL , 1.794 mmol) and 2-cyanoethyl-N,N-Diisopropylchlorophosphoramidite (56.8 μL , 0.255 mmol) were added and the solution stirred for 11 h at room temperature. The solvents were evaporated in vacuo, CH_2Cl_2 was added, the solution was poured into aqueous saturated NaHCO_3 solution and extracted with CH_2Cl_2 (2 x). The combined organic layers were dried over anhydrous Na_2SO_4 , the solvent was evaporated and the remaining solid was purified by flash chromatography on silica gel (Petrolether/EtOAc 2:1 + 1 % NEt_3). Following, the product was dissolved in benzene (3 mL) and lyophilized to yield the title compound (119 mg, 95 %) as pale purple foam, which was dissolved in dry MeCN and applied directly for oligonucleotide synthesis. Instead of lyophilization, precipitation of the product from a solution in EtOAc by addition of hexane under vigorous stirring at $-50\text{ }^\circ\text{C}$ gave consistent results. $R_f = 0.76$ (Hexane/EtOAc 1:1 + 1 % NEt_3)

^{31}P NMR (121 MHz, CDCl_3): $\delta = 149.9, 149.8$ - MS (ESI, DCM/MeOH + 10 mmol/L NH_4Ac): m/z (%) = 986.5 (62) $[\text{MH}^+]$, 303.2 (100) $[\text{DMT}^+]$

5'-O-(tetraisopropylidisiloxane-1,3-diyl)-uridine

A flask was charged with dry pyridine (75.0 mL) and uridine (3.629 g, 14.86 mmol). 1,3-dichloro-1,1,3,3-tetraisopropylidisiloxane (5.23 mL, 16.35 mmol) was added under nitrogen atmosphere and the reaction mixture was stirred at room temperature for 6 hours, followed by addition of EtOAc and aqueous saturated NaHCO₃ solution. After stirring for 30 minutes, the mixture was poured into EtOAc, separated and the aqueous layer was extracted with EtOAc. The organic layers were pooled and dried over anhydrous MgSO₄. The solvent was removed under reduced pressure and the crude product was purified by gradient flash chromatography on silica gel (CH₂Cl₂/acetone 6:1 to 3:1) to yield 5'-O-(tetraisopropylidisiloxane-1,3-diyl)-uridine as a colorless foam (5.778 g, 80 %).

The analytical data was consistent with the literature.^[31]

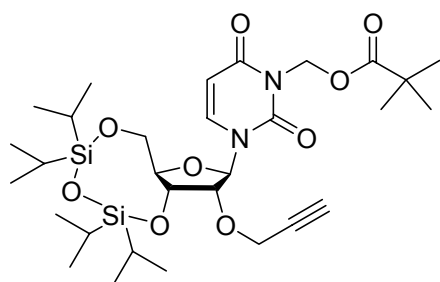
3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-N³-pivaloyloxymethyl-uridine

To a solution of 5'-O-(tetraisopropylidisiloxane-1,3-diyl)-uridine (5.726 g, 11.78 mmol) in CH₂Cl₂ (250 mL), a solution of Na₂CO₃ (250 mL, 120 mmol) and tetrabutylammonium hydrogensulfate (1.004 g, 2.96 mmol) were added. The mixture was vigorously stirred at room temperature for 10 minutes, and pivaloyl chloride (14.51 mL, 117.81 mmol) was added

in one portion. The reaction mixture was stirred at room temperature for 48 hours, and was extracted with CH_2Cl_2 (2 x 200 mL). The aqueous layer was extracted with Et_2O (2 x 100 mL), the organic layers were pooled and concentrated to half volume, dried over anhydrous Na_2SO_4 and the solvents were removed under reduced pressure. Purification was performed by gradient flash chromatography on silica gel ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 80:1 to 20:1), followed by trituration with a $\text{Et}_2\text{O}/\text{CH}_2\text{Cl}_2$ mixture over night to yield 3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)- N^3 -pivaloyloxymethyl-uridine as glistening colorless foam (4.949 g, 70 %).

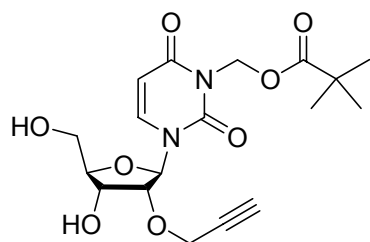
The analytical data was consistent with the literature.^[31]

3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)- N^3 -pivaloyloxymethyl-2'-O-propargyluridine



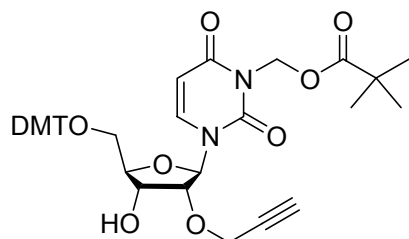
A solution of 3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)- N^3 -pivaloyloxymethyl-uridine (4.890 g, 8.15 mmol) in dry MeCN (50 mL) was cooled to 0 °C. Propargyl bromide (945 μL , 10.60 mmol) and 2-tert-butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorine (2.95 mL, 10.19 mmol) were added under nitrogen atmosphere and the reaction mixture was stirred at 0 °C for 30 minutes. Following, it was stirred at ambient temperature for 90 minutes, the solvent was evaporated under reduced pressure and dried over night in vacuo. The raw product was purified by gradient flash chromatography on silica gel ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 200:1 to 100:1). Evaporation of the solvent yielded 3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)- N^3 -pivaloyloxymethyl-2'-O-propargyl-uridine as colorless foam (1.560 g, 30 %).

The analytical data was consistent with the literature.^[31]

***N*³-pivaloyloxymethyl-2'-O-propargyluridine**

To a solution of 3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-*N*³-pivaloyloxymethyl-2'-O-propargyluridine (1.499 g, 2.35 mmol) in dry THF (25 mL), there was added NEt₃·3HF (1.53 mL, 9.39 mmol). This mixture was stirred at ambient temperature for 16 hours. Following, the mixture was poured into brine/CH₂Cl₂ (80 mL, v/v 1:1), separated and the aqueous layer was extracted with EtOAc (2 x 40 mL). The organic layers were pooled and dried over anhydrous Na₂SO₄. The solvents were removed under reduced pressure and the residue was purified by flash chromatography on silica gel (CH₂Cl₂/MeOH 8:1) to afford *N*³-pivaloyloxymethyl-2'-O-propargyluridine as a colorless foam (642 mg, 69 %).

The analytical data was consistent with the literature.^[31]

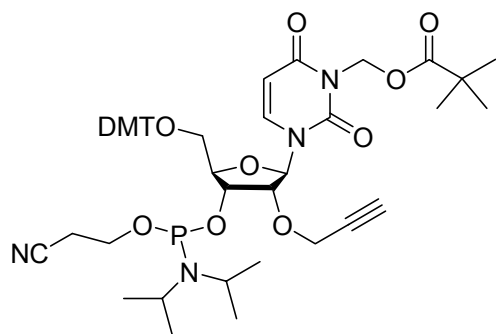
5'-O-dimethoxytrityl-*N*³-pivaloyloxymethyl-2'-O-propargyluridine

To a well stirred solution of *N*³-pivaloyloxymethyl-2'-O-propargyluridine (537 mg, 1.36 mmol) in dry pyridine (18.0 mL) there was added 4,4'-dimethoxytriphenylmethyl chloride (551 mg, 1.63 mmol), followed by dry NEt₃ (596 μL, 4.28 mmol) under nitrogen atmosphere. The reaction mixture was stirred for 16 hours at room temperature in the dark. The reaction mixture was evaporated to dryness to afford mucous orange oil. Following, the crude product was dissolved in a mixture of CH₂Cl₂ and brine. After separation, the aqueous layer was

extracted with EtOAc and the combined organic layers were dried over anhydrous Na_2SO_4 . The solvents were evaporated under reduced pressure and purification by gradient flash chromatography on silica gel (Hexane/EtOAc 3:1 + 1 % NEt_3 to 2:1 + 1 % NEt_3) gave 5'-O-dimethoxytrityl- N^3 -pivaloyloxymethyl-2'-O-propargyluridine as glistening white foam (873 mg, 92 %). $R_f = 0.15$ (Hexane/EtOAc 3:1 + 1 % NEt_3)

The analytical data was consistent with the literature.^[31]

5'-O-dimethoxytrityl- N^3 -pivaloyloxymethyl-2'-O-propargyluridine-3'-O-(2-cyanoethyl-N,N-diisopropylphosphoramidite)



5'-O-dimethoxytrityl- N^3 -pivaloyloxymethyl-2'-O-propargyluridine (200 mg, 0.287 mmol) was co-evaporated with anhydrous acetonitrile (2 x) and dry CH_2Cl_2 (8.0 mL) was added. Anhydrous NEt_3 (250 μL , 1.794 mmol) was added and the solution was degassed. Following, 2-cyanoethyl-N,N-Diisopropylchlorophosphoramidite (141 μL , 0.632 mmol) was added under argon atmosphere and the solution stirred for 4 h at room temperature. Since TLC did not show complete reaction, additional NEt_3 (250 μL , 1.794 mmol) and 2-cyanoethyl-N,N-Diisopropylchlorophosphoramidite (1.0 eq.) were added. The reaction mixture was stirred at ambient temperature for 1 hour. Next, the solvents were evaporated under reduced pressure, CH_2Cl_2 was added to the residue and the solution was poured into aqueous saturated NaHCO_3 solution. The organic layer was dried over anhydrous Na_2SO_4 , the solvent was evaporated and the remaining solid was purified by flash chromatography on silica gel (Petrolether/EtOAc 3:2 + 1 % NEt_3). The pure fractions were concentrated under reduced pressure, then dissolved in EtOAc and hexane was added under vigorous stirring at $-50\text{ }^\circ\text{C}$ to precipitate the title compound as colorless crystals (240 mg, 93 %). $R_f = 0.32$ (Hexane/EtOAc 3:2 + 1 % NEt_3)

The analytical data was consistent with the literature.^[31]

3.5. References

- [1] a)U. Pischel, *Angew. Chem., Int. Ed.* **2007**, *46*, 4026; b)F. R. Raymo, M. Tomasulo, *Chem. Eur. J.* **2006**, *12*, 3186.
- [2] a)G. Mayer, A. Heckel, *Angew. Chem. Int. Ed. Engl.* **2006**, *45*, 4900; b)D. Vomasta, C. Högner, N. R. Branda, B. König, *Angew. Chem. Int. Ed. Engl.* **2008**, *47*, 7644.
- [3] C. Renner, L. Morode, *ChemBioChem* **2006**, *7*, 868.
- [4] a)X. Liang, N. Takenaka, H. Nishioka, H. Asanuma, *Chem. Asian J.* **2008**, *3*, 553; b)GlenResearch, *GlenReports* **2009**, *21*, 7.
- [5] a)H. Nishioka, X. Liang, H. Kashida, H. Asanuma, *Chem. Commun.* **2007**, 4354; b)A. Yamazawa, X. Liang, H. Asanuma, M. Komiyama, *Angew. Chem. Int. Ed. Engl.* **2000**, *39*, 2356; c)X. Liang, H. Asanuma, M. Komiyama, *J. Am. Chem. Soc.* **2002**, *124*, 1877; d)H. Asanuma, X. Liang, H. Nishioka, D. Matsunaga, M. Liu, M. Komiyama, *Nat. Protoc.* **2007**, *2*, 203.
- [6] a)A. Deiters, *ChemBioChem* **2009**, *11*, 47; b)H. Lusic, R. Uprety, A. Deiters, *Org. Lett.* **2010**, *12*, 916.
- [7] a)S. Ogasawara, M. Maeda, *Angew. Chem., Int. Ed.* **2008**, *47*, 8839; b)S. Ogasawara, H. Maeda, *Angew. Chem. Int. Ed. Engl.* **2009**, *1*; c)Y. Saito, K. Matsumoto, Y. Takeuchi, S. S. Bag, S. Kodate, T. Morii, I. Saito, *Tetrahedron Lett.* **2009**, *50*, 1403.
- [8] G. Berkovic, V. Krongauz, V. Weiss, *Chem. Rev.* **2000**, *100*, 1741.
- [9] a)D. D. Young, A. Deiters, *ChemBioChem* **2008**, *9*, 1225; b)J. Andersson, S. Li, P. Lincoln, J. Andreasson, *J. Am. Chem. Soc.* **2008**, *130*, 11836; c)R. Dalton, M. O. Stone, E. Heckman, J. A. Hagen, N. Ogata, F. K. Hopkins, J. S. Zetts, R. L. Nelson, D. E. Diggs, J. G. Grote, *Mol. Cryst. Liq. Cryst.* **2005**, *426*, 3.
- [10] J. Andreasson, J. Andersson, P. Lincoln, S. Li, *Nucleic Acids Symp. Ser.* **2008**, 675.
- [11] T. Sakata, Y. Yan, G. Marriott, *J. Org. Chem.* **2005**, *70*, 2009.
- [12] N. Angelini, B. Corrias, A. Fissi, O. Pieroni, F. Lenci, *Biophys. J.* **1998**, *74*, 2601.
- [13] A. Koçer, M. Walko, W. Meijberg, B. L. Feringa, *Science* **2005**, 755.
- [14] a)H. Asanuma, K. Shirasuka, T. Yoshida, T. Takarada, X. Liang, M. Komiyama, *Chem. Lett.* **2001**, 108; b)P. Zhang, J. B. Meng, T. Matsuura, Y. M. Wang, *Chin. Chem. Lett.* **2002**, *13*, 299.

- [15] a)H. Kashida, T. Fujii, H. Asanuma, *Org. Biomol. Chem.* **2008**, 2892; b)R. Huber, N. Amann, H.-A. Wagenknecht, *J. Org. Chem.* **2004**, 69, 744; c)D. Baumstark, H.-A. Wagenknecht, *Angew. Chem. Int. Ed. Engl.* **2008**, 47, 2612.
- [16] N. Amann, R. Huber, H.-A. Wagenknecht, *Angew. Chem., Int. Ed.* **2004**, 43, 1845.
- [17] a)N. Amann, H.-A. Wagenknecht, *Tetrahedron Lett.* **2003**, 44, 1685; b)P. M. E. Gramlich, S. Warncke, J. Gierlich, T. Carell, *Angew. Chem., Int. Ed.* **2008**, 47, 3442; c)M. K. Schlegel, L.-O. Essen, E. Meggers, *J. Am. Chem. Soc.* **2008**, 130, 8158; d)V. V. Filichev, E. B. Pedersen, *J. Am. Chem. Soc.* **2005**, 127, 14849; e)S. Berndl, N. Herzig, P. Kele, D. Lachmann, X. Li, O. S. Wolfbeis, H.-A. Wagenknecht, *Bioconjugate Chem.* **2009**, 20, 558.
- [18] a)L. Zhang, A. Peritz, E. Meggers, *J. Am. Chem. Soc.* **2005**, 127, 4174; b)L. Zhang, A. E. Peritz, P. J. Carroll, E. Meggers, *Synthesis* **2006**, 645.
- [19] I. Géci, V. V. Filichev, E. B. Pedersen, *Chem. Eur. J.* **2007**, 13, 6379.
- [20] C. Wanninger, H.-A. Wagenknecht, *Synlett* **2006**, 2051.
- [21] G. W. Kabalka, M. Varma, R. S. Varma, *J. Org. Chem.* **1986**, 51, 2386.
- [22] G. T. Hermanson, *Bioconjugate Techniques* **1996**, Academic Press.
- [23] B. Sammet, *Synlett* **2009**, 3050.
- [24] a)K.-Y. Tomizaki, X. Jie, H. Mihara, *Bioorg. Med. Chem. Lett.* **2005**, 15, 1731; b)K.-Y. Tomizaki, H. Mihara, *J. Mater. Chem.* **2005**, 2732.
- [25] a)S. Berndl, H.-A. Wagenknecht, *Angew. Chem. Int. Ed. Engl.* **2009**, 48, 2418; b)F. Menacher, M. Rubner, S. Berndl, H.-A. Wagenknecht, *J. Org. Chem.* **2008**, 73, 4263.
- [26] GlenResearch, *Glen Reports* **2010**, 22, 6.
- [27] M. Alvira, S. J. Quinn, A. Avino, D. Fitzmaurice, R. Eritja, *Open Org. Chem. J.* **2008**, 2, 41.
- [28] S. H. Weisbrod, A. Marx, *Chem. Commun.* **2008**, 5675.
- [29] E. M. Sletten, C. R. Bertozzi, *Angew. Chem. Int. Ed. Engl.* **2009**, 48, 6974.
- [30] P. M. E. Gramlich, C. T. Wirges, A. Manetto, T. Carell, *Angew. Chem., Int. Ed.* **2008**, 47, 8350.
- [31] a)M. Grotli, M. Douglas, R. Eritja, B. S. Sproat, *Tetrahedron* **1998**, 5899; b)M. Grotli, R. Eritja, B. S. Sproat, *Tetrahedron* **1997**, 53, 11317.
- [32] a)A. K. Chibisov, H. Görner, *J. Phys. Chem. A* **1997**, 101, 4305; b)H. Görner, *Chem. Phys.* **1997**, 222, 315.

Chapter 4:

An alternative postsynthetic methodology for DNA labeling and new compositions of versatile building blocks for oligonucleotide chemistry

4.1. Introduction

4.1.1. DNA labeling

Three generic synthetic approaches serve for the directed covalent modification of oligonucleotides. First of all, the triphosphates of appropriate compounds can be synthesized and then incorporated with use of polymerases into DNA.^[1] Second, the desired DNA modifications can be prepared separately with use of phosphoramidite chemistry and incorporated into DNA on solid support as subset building blocks using a DNA synthesizer (Scheme 1.1, Route a).^[2] However, if by any means the phosphoramidite of the desired label or probe is hardly accessible due to synthetic challenges that may arise from its instability in terms of DNA synthesis conditions, a third method can be selected (Scheme 1.1, Route b).

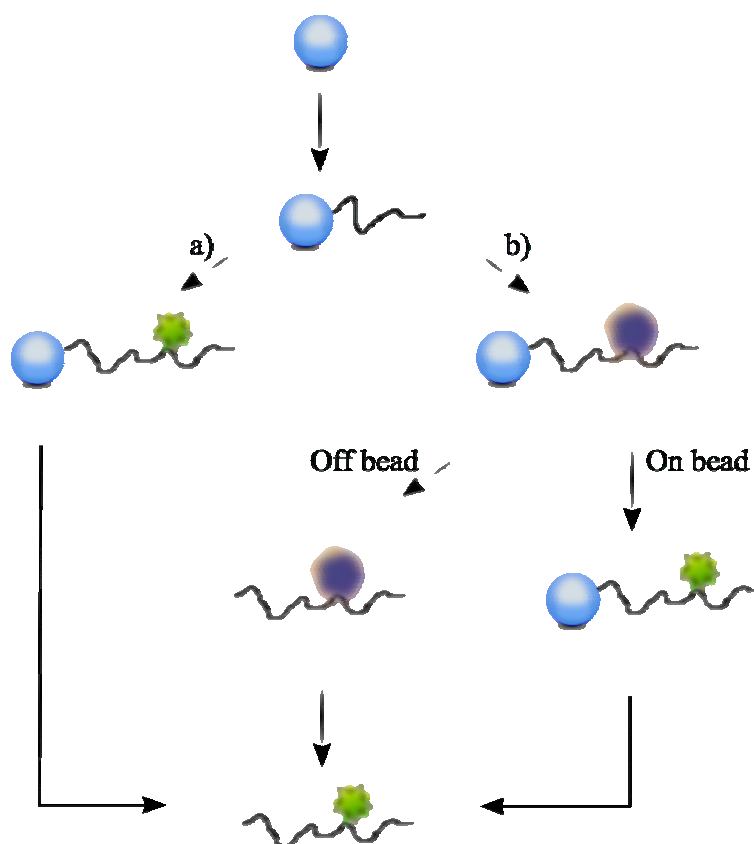


Figure 1.1. Schematic illustration for directed synthesis of modified oligonucleotides using phosphoramidite and postsynthetic pathways

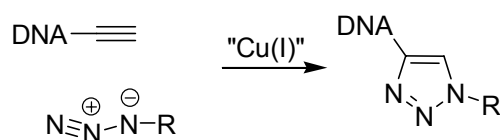
4.1.2. Postsynthetic methods

This method can generally be described as a postsynthetic approach,^[3] although it fans out to different techniques. A subdivision can be made with respect towards the time when the modification is introduced into the DNA; either if the synthesized oligonucleotide is still attached to the CPG (“On bead labeling”) or if the label is linked after deprotection and cleavage of the oligonucleotide to the latter (“Off bead labeling”).

These subdivisions can be further discriminated, particularly by taking temporal aspects into account, e.g., successive introduction of several labels using the phosphoramidite method has been described,^[4] as well as blending with sequential on bead Pd-catalyzed Sonogashira cross-coupling reactions with ethynylpyrene.^[5] On and off bead labeling was expanded by use of specific deprotection steps that can be used to label oligonucleotides via modular protocols.^[6] Although, it is not illustrated in particular in Figure 1.1, it should also be mentioned that utilization of the temporal final detritylation step can offer further advances, especially with regard to purification and sample workup.^[2c, 7]

4.1.3. CuAAC for DNA labeling

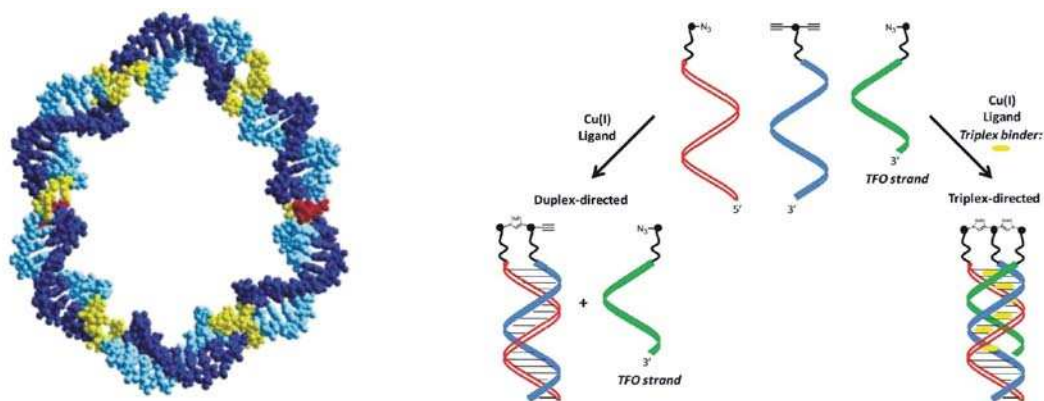
However, the postsynthetic modification of oligonucleotides allows the covalent linkage of molecules in a bioorthogonal fashion. Since alkyne and azide groups typically are not present in oligonucleotides and react regioselectively under Cu(I) catalysis by regioselective formation of 1,4-substituted 1,2,3-triazoles,^[8] the click reaction (CuAAC) matches the requirements for bioorthogonality.^[9] Further details on CuAAC are introduced and discussed in Chapter 2.



Scheme 1.1. CuAAC of DNA and azide compound

Especially for labeling and modification of DNA, click chemistry depends on an azide group of the modifier that reacts with a presynthesized oligonucleotide carrying terminal alkyne groups.^[6, 10] With this approach the time consuming synthesis of phosphoramidites as DNA building blocks can be avoided. Interestingly, off bead click chemistry has been used for efficient and rapid interstrand cross-linking,^[11] intrastrand circularization and catenation^[12] as well as directed formation of either duplex or triplex DNA.^[13] Also, 5'-functionalizations of oligonucleotides on solid support into azides^[14] as well as microwave-assisted Cu(I)-catalyzed

cycloadditions using postsynthetic off bead labeling have been reported.^[15] The merging of 5'-azidation and CuAAC assisted by microwaves has culminated in new strategies for the synthesis of oligonucleotides incorporating alkyne-groups and galactosyl azide derivatives^[16] as well as cyclic and bicyclic oligonucleotides.^[17]



Scheme 1.2. DNA-directed CuAAC reactions. DNA circularization and catenation^[12] (left) and control of duplex and triplex DNA (right).^[13]

However, the published click-type modifications of oligonucleotides with functional π -systems are limited to reactions between azide groups as part of the modifying molecule and acetylene groups as part of the oligonucleotide.^[10, 18] Remarkably, this methodology has been successfully commercialized by baseclick GmbH, and Invitrogen Corporation within the framework of Click-iT® detection assays using EdU and fluorescent azides.^[19]

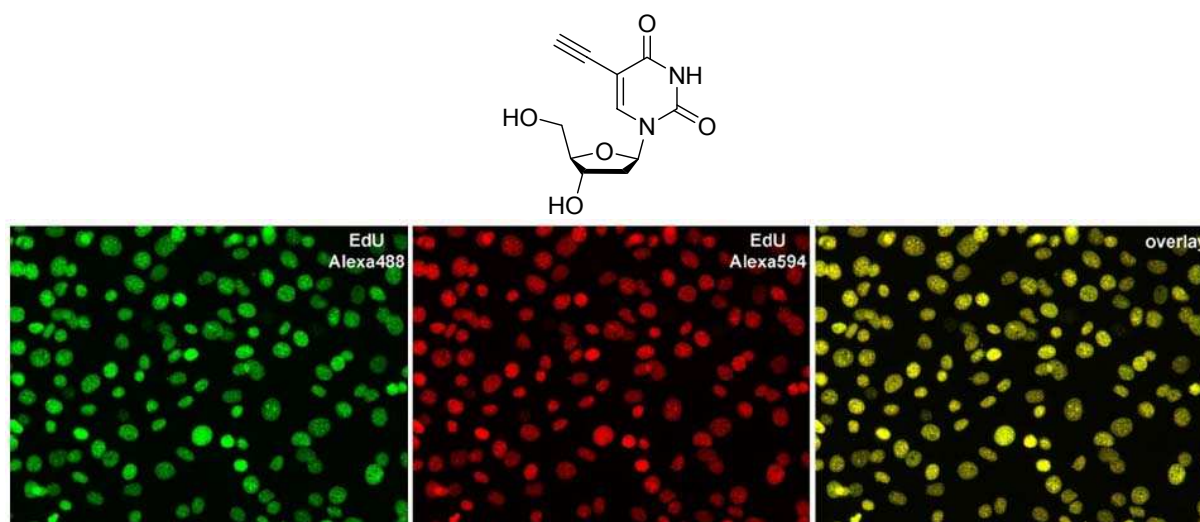


Figure 1.2. EdU (top), and fluorescence microscopy images (bottom) of NIH3T3 cells labeled with EdU and successively reacted with Alexa488 and Alexa594-azide.^[19]

Based on the aforementioned methodologies for DNA modification, we present three different approaches. Foremost, first examples of a new, alternative postsynthetic method are expounded: this on bead labeling system allows the click reaction of ethynyl-modified labels with an azide group in the 5-position of 2'-deoxyuridine that was formed in situ from a presynthesized oligonucleotide in one step. We also present the synthesis of a quinolinol derivative as potential metallobase nucleic acid modification and preliminary results after successful click conjugation to DNA by using off bead labeling. In the last section, the synthesis and optical properties of a new photoswitchable spirobenzopyran nucleoside that can be used for the triphosphate approach are showcased.

4.2. Results and Discussion

4.2.1. An alternative postsynthetic route for DNA labeling

Since the demand for labeled oligonucleotides is rapidly growing, we wanted to develop a new alternative method for labeling DNA that would expand the use of the bioorthogonal click strategy. Therefore, it seemed to be reasonable to upturn the existing methods that have been described before. This complementary access would then complete the postsynthetic repertoire of labeling techniques involving CuAAC. In our opinion, a very good and reasonable way to parlay this method was to use a postsynthetic approach involving on bead labeling due to the advances solid-phase synthesis entails (e.g., excess of reagents, easy washing, unified manipulation of small quantities) and to circumvent chemical stability issues of the azides during DNA synthesis and workup.^[20]

4.2.1.1. Synthesis of nile red and 3-Ethynylperylene

To test the functionalization of oligonucleotides with our reverted click approach, we chose 2-Ethynyl nile red **1** and 3-Ethynylperylene **2** as chromophores (Figure 2.1). They were prepared in five and three steps with an overall yield of 39 %, and 88 % respectively. The synthetic steps are discussed in detail in Chapter 2.

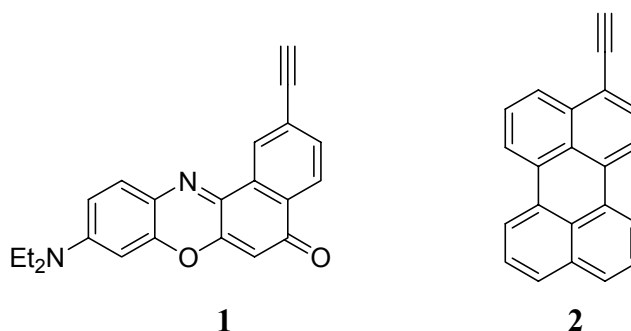
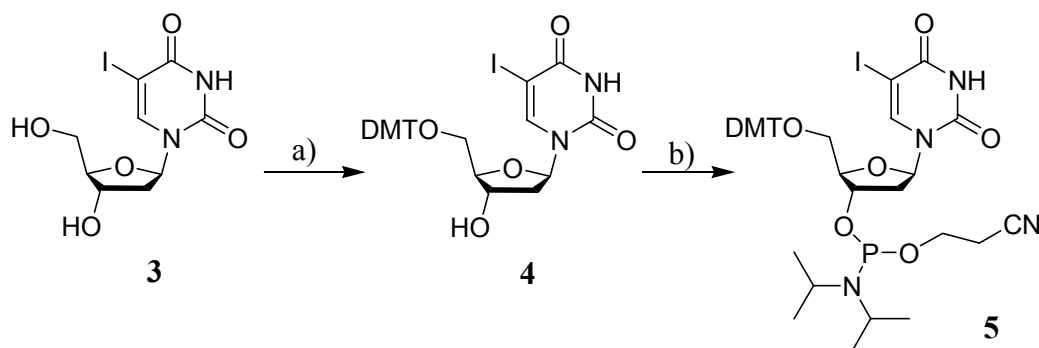


Figure 2.1. 2-Ethynyl nile red and 3-Ethynylperylene

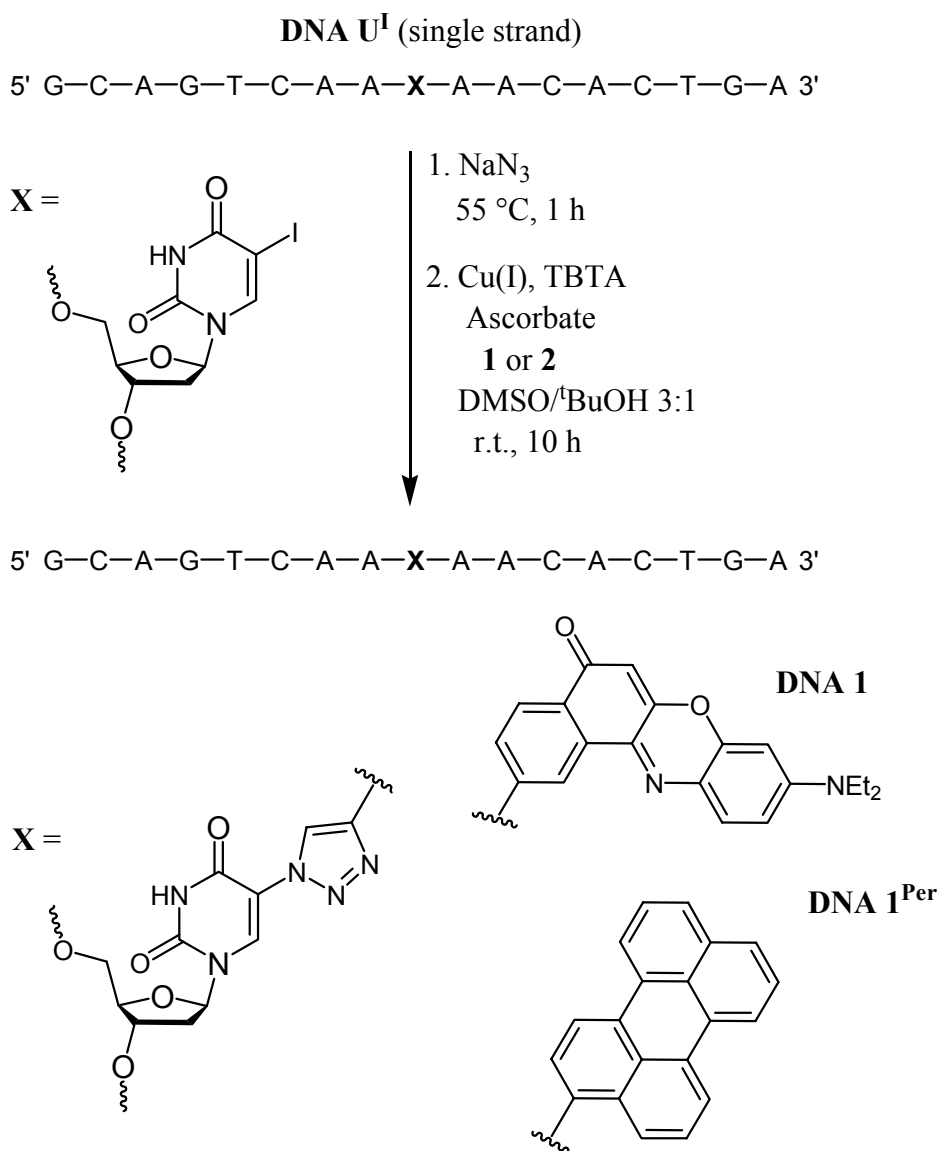
4.2.1.2. In situ azide formation and click reaction

At the beginning the procedure starts with the synthesis of the oligonucleotide for **DNA U^I**, bearing 5-iodo-2'-deoxyuridine (IdU) in the middle of the sequence. Although, the respective phosphoramidite of IdU is commercially available, it can be synthesized in two synthetic steps, starting from IdU **3**. Protection at the 5'-OH position with use of a standard protocol afforded the tritylated compound **4** in 94 % yield. In the next step, phosphitylation of **4** with 2-cyanoethyl-N,N-diisopropylchlorophosphoramidite gave compound **5** as the desired phosphoramidite building block.



Scheme 2.1. Synthesis of 5-IdU-Phosphoramidite **5**. Reagents and conditions: a) DMTCI (1.2 eq.), NEt₃ (3.1 eq.), pyridine, r.t., 24 h, 94 %; b) **23** (3.3 eq.), NEt₃ (14.1 eq.), CH₂Cl₂, r.t., 1 h, 92 %.

However, **5** was introduced into DNA by using standard coupling conditions (Scheme 2.2, **DNA U^I**). Subsequently, the oligonucleotide (1 μmol) was treated on solid support with an excess of sodium azide (200 μmol) in DMSO to yield in situ the 5-azidoooligonucleotide which has been described as photoaffinity label.^[20a, 21] After the nucleophilic displacement of the iodine group by azide, a washing step was performed, and the azido-oligonucleotide was coupled with the ethynyl nile red compound **1**, or perylene derivative **2** respectively, in the presence of Cu(I).



Scheme 2.2. Postsynthetic protocol for the preparation of the nile red modified **DNA 1** and Perylene modified **DNA 1^{Per}**

At this point the boon of our alternative approach is evident: an additional and outstanding benefit of click chemistry on solid phase is that conjugates can be prepared on the synthesis columns (or Eppendorf cups and assimilable devices) so that excess of expensive acetylene tags can be readily recovered prior to oligonucleotide deprotection. Finally, the oligonucleotides were cleaved from the solid support and deprotected under very mild conditions (NH₄OH, r.t., 18 h), desalted and analyzed by HPLC and mass spectrometry.

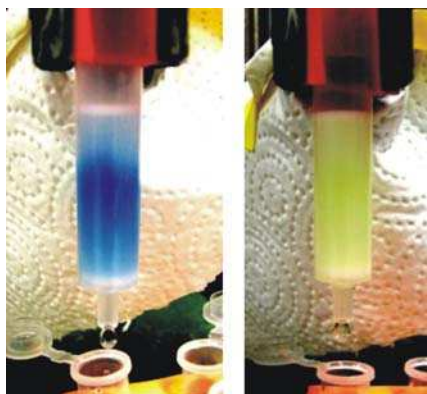


Image 2.1. Visual representation of Sephadex gel filtration of **DNA 1** (left) and **DNA 1^{Per}** (right).

Interestingly, the overall yield for the postsynthetic ligation to **DNA 1** is 10 % compared to the phosphoramidite building block approach that was used for **DNA 2** in comparison,^[4a, 22] whereas the amount of purified perylene labeled **DNA 1^{Per}** was sufficient enough for detection with HPLC-DAD (Figure 2.2) where two distinctive absorption bands of the conjugated perylene moiety are observable ($\lambda_{\text{abs}} = 431$ and 457 nm). Regardless, the successful conjugation of nile red **1** and perylene **2** to the oligonucleotides was also verified by ESI mass spectrometry.

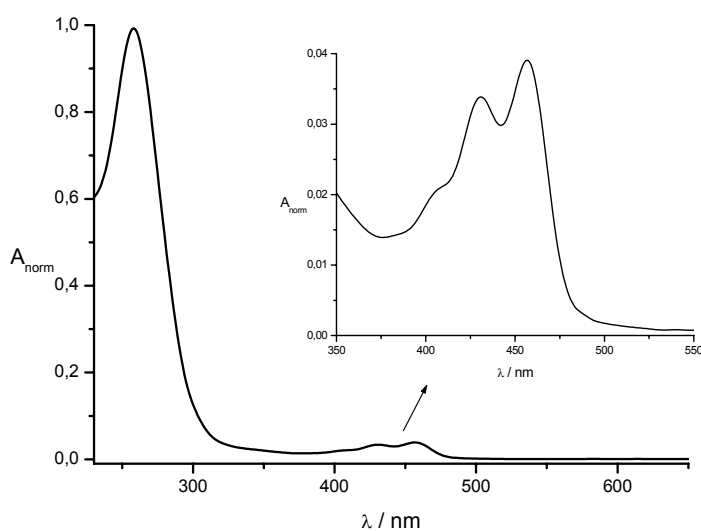
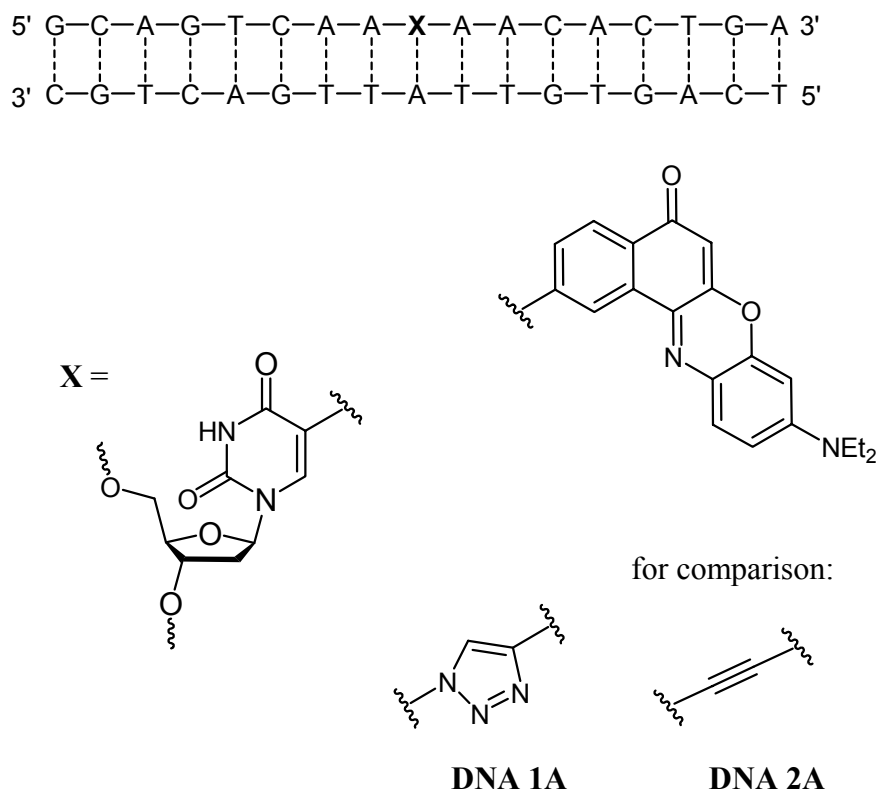


Figure 2.2. Normalized UV/Vis absorption spectrum of **DNA 1^{Per}** (absorbance during HPLC 25.29-26.26 min).

4.2.1.3. Optical properties of nile red labeled DNA



Scheme 2.3. Comparison of duplexes **DNA 1A** and **DNA 2A**.

Both, the single stranded **DNA 1** and the duplex **DNA 1A** show clearly the presence of the covalently attached nile red chromophore by a peak at ca. 610 nm. By excitation at 610 nm the steady state fluorescence exhibits a broad signal with a maximum at 658 nm and a quantum yield of 29 % in the single strand and 31 % in the duplex. It is interesting to observe that the optical properties of **DNA 1A** are very similar in comparison with **DNA 2A**^[4a] bearing the nile red dye attached via an ethynyl linker to 2'-deoxyuridine (Table 2.1). Remarkably, the quantum yield of **DNA 1A** is significantly higher than that of **DNA 2A**.

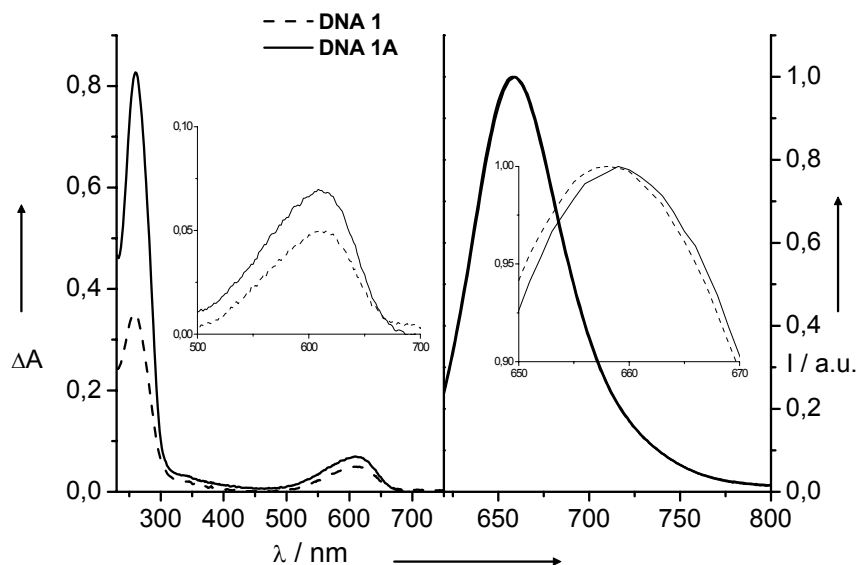


Figure 2.3. UV/Vis absorption spectra (left) and fluorescence spectra (right) of the single-stranded **DNA 1** and the corresponding double-stranded **DNA 1A**, each 2.5 μM in NaP_i buffer (10 mM), NaCl (250 mM), $\lambda_{\text{exc}} = 610$ nm.

The melting temperature of **DNA 1A** is 58.1 $^{\circ}\text{C}$, whereas the thermal stability for **DNA 2A** is slightly lower (57.1 $^{\circ}\text{C}$). Compared to the unmodified duplex ($X = \text{T}$), the Nile Red modified duplex **DNA 1A** including the triazolyl liker destabilizes the duplex by 2.1 $^{\circ}\text{C}$, whereas **DNA 2A** destabilizes the duplex by 3.6 $^{\circ}\text{C}$. This indicates that the aromatic triazolyl bridge of the nile red conjugation in **DNA 1A** is able to regain more of the lost thermal stability by aromatic interactions with the adjacent base pairs.

	DNA 1A	DNA 2A ^[4a]
λ_{max} (Absorption)/nm	612 (ss) 608 (ds)	615 (ss) 615 (ss)
$\epsilon/10^4 \text{ M}^{-1}\text{cm}^{-1}$	2.0 (ss) 2.8 (ds)	2.6 (ss) 2.5 (ds)
λ_{max} (Emission)/nm	658 (ss) 659 (ds)	658 (ss) 665 (ds)
$\Phi_{\text{F}}^{\text{a}}$	0.29 (ss) 0.30 (ds)	0.20 (ss) 0.10 (ds)
$T_{\text{m}}^{\text{b}}/^{\circ}\text{C}$ ($\Delta T_{\text{m}}/^{\circ}\text{C}$) ^c	58.1 (-2.6)	57.1 (-3.6)

Table 2.1. Comparison of the optical data of **DNA 1A** and **DNA 2A**. ^a Determined with cresyl violet as the standard, $\Phi_{\text{F}} = 0.54$; ^b T_{m} measured at 260 nm; ^c In comparison to unmodified reference duplex ($X = \text{T}$), $T_{\text{m}} = 60.7$ $^{\circ}\text{C}$.

Moreover, we carried out calculations of the LUMOs of both Nile red modified nucleosides. For geometry optimization, calculations using MMFF were performed that minimize distortion from parameterized ideal bond distances and angles. MMFF also was used to optimize geometry with respect to Coulombic and non-bonded van der Waals interactions. After full geometry optimization, the LUMOs were then calculated with use of the semi-empirical AM1 method. Interestingly, since the linkage of the fluorophore to the oligonucleotide is a critical issue for the resulting optical properties, the results of the calculations indicate that both bridges lead to largely delocalized orbitals and to exciplex-like states that show unstructured, solvent-dependent fluorescent bands.

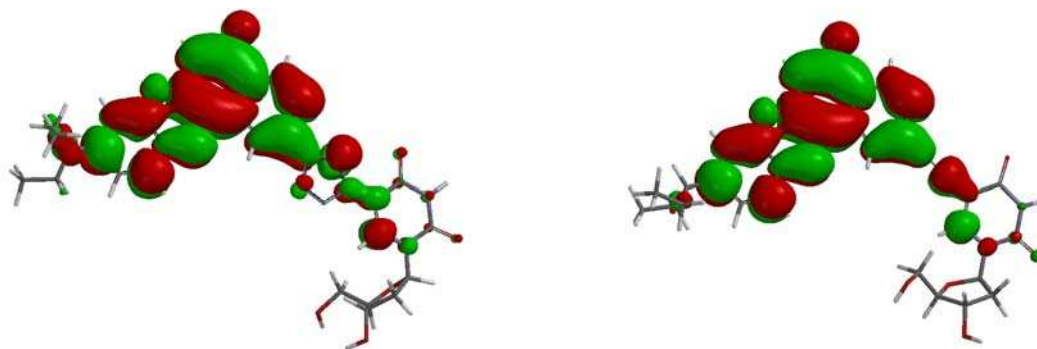
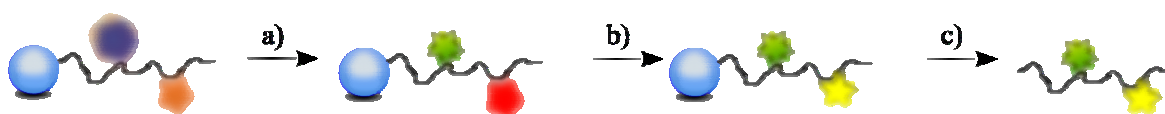


Figure 2.4. Calculated LUMOs for the isolated Nile red modified 2'-deoxyuridine of **DNA 1A** (left) and **DNA 2A** (right).

Further investigations with respect towards improved azidification yields and cleavage conditions are performed at present. Nevertheless, the method showcased herein can be further expanded as illustrated in Scheme 2.4. There, an oligonucleotide is equipped with building blocks bearing acetylene and iodo groups. The oligonucleotide is then subjected under typical click conditions with the desired labeling molecule bearing an azide group, followed by straightforward in situ azide formation (Step a). In the next step the azide is subjected with an acetylene modified label, again under click conditions on solid support (Step b). Final deprotection then affords the sequentially labeled oligonucleotide (Step c).



Scheme 2.4. Illustration of sequential click labeling

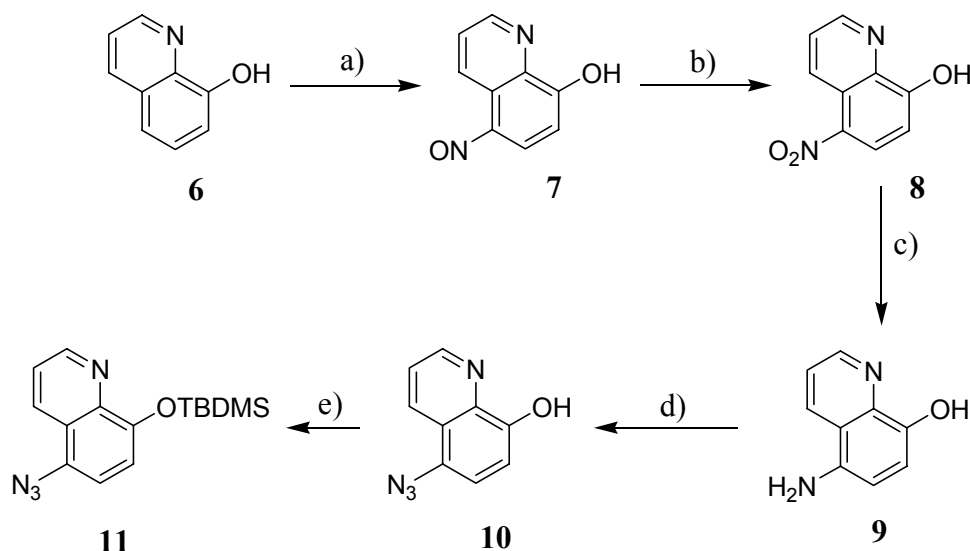
4.2.2. Enhancements for the synthesis of metal-containing DNA by postsynthetic off bead labeling with quinolinol

Since the incorporation of metal complexes into oligonucleotides is a key design target for the functionalization of DNA creating new binding motifs in duplex DNA,^[23] our approach focused on the incorporation of a bidentate quinolinol moiety. These metallobases are expected to be used for the control of distinct properties in DNA through the addition or removal of selected metal ions, e.g. two 8-hydroxyquinoline ligands can coordinate a central Cu^{2+} ion in a square planar fashion.^[24] Specifically, our approach involves the synthesis of quinolinol derivatives bearing azido-groups, followed up by CuAAC with an alkyne-bearing oligonucleotide using off bead labeling.

4.2.2.1. Synthesis of Azido-Quinolinol

Being an exceptionally strong bidentate ligand for various transition metal ions and due to its extended hydrophobic aromatic surface,^[24] quinolinol was chosen as a promising candidate for prospective metal-mediated interstrand binding studies. E.g., charge transfer processes have been described with 8-hydroxyquinoline and its derivatives, chelating Cu(II) , Ni(II) and Zn(II) ^[25] and are therefore also of interest for oligonucleotide chemistry.

5-nitroquinolinol can be prepared by direct nitration of 8-hydroxyquinoline **6** using nitrating mixture, but the reaction results in a mixture of the desired product, its 7-nitro isomer and the 5,7-dinitro derivative.^[26] Thus, a two step method for the synthesis of the nitro compound was chosen. Therefore, nitrosation of compound **6** using sodium nitrite in dilute sulphuric acid afforded **7** in excellent yield.^[27] Subsequently, oxidation of **7** gave the nitro compound **8** in good yield (81 %) as a brown-yellow solid.^[28] The aromatic amine **9** was then synthesized by hydrogenation of the nitro group. Therefore, **8** was reduced in a mixture of ethyl acetate and methanol using palladium on activated charcoal under H_2 atmosphere. Addition of Celite and use of EDTA as a competing ligand then helped to efficiently remove the catalyst from the reaction mixture, and compound **9** was obtained in 95 % yield. Diazotization of **9** followed by reaction with sodium azide in situ gave compound **10** after simple extraction with diethyl ether in 30 % yield, similar to the literature (32 %).^[29] To prevent complexation of copper ions during click synthesis we additionally protected 5-azido-quinolinol **10** in the last step as TBDMS ether by treatment with 1.2 eq. TBDMSCl in the presence of 1.4 eq. imidazole, leading to compound **11** in 87 % yield.



Scheme 2.5. Synthesis of Azido-Quinolinol. Reagents and conditions: a) NaNO_2 (1.1 eq.), H_2O , H_2SO_4 , 95 %; b) NaOH (1.3 eq.), H_2O_2 (1.5 eq.), 70 °C, 1 h, 81 %; c) H_2 , Pd/C, MeOH/EtOAc, 95 %; d) NaNO_2 (1.9 eq.), H_2O , HCl, NaN_3 (2.4 eq.), 3 h, 3 °C to r.t., 18 h, 30 %; e) TBDMSCl (1.2 eq.), imidazole (1.4 eq.), CH_2Cl_2 , r.t., 69 h, 87 %.

4.2.2.2. Click reaction of Azido-quinolines with alkyne-modified oligonucleotides

In order to prepare the quinolinol-conjugated oligonucleotides we performed CuAAC with both quinolinol azides **10** and **11** and presynthesized DNA bearing the terminal alkyne group. The alkyne component in the DNA was inserted by using the 2'-propargyl modified building block **12** that was prepared and incorporated into DNA as described in Chapter 3. For effective incorporation of the phosphoramidite **12**, the concentration was increased (0.1 M) and the coupling time was extended (6.3 min). After preparation, the oligonucleotide was cleaved from the resin and deprotected by treatment with conc. NH_4OH at room temperature for 22 hours.

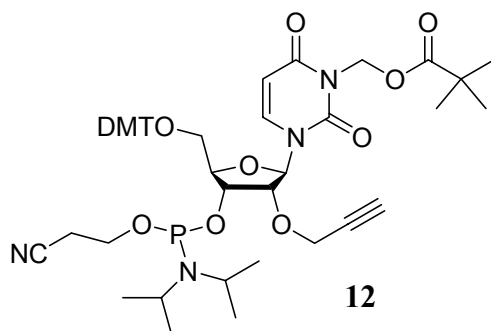
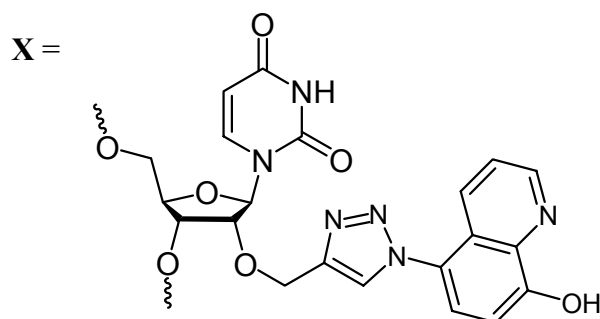
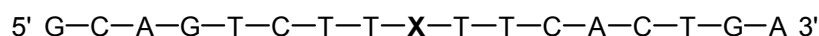


Figure 2.5. Building block for DNA click reactions.

For the click ligations the presynthesized oligonucleotides were treated with azide **10** or **11** in the presence of Cu(I), TBTA and sodium ascorbate in a solvent mixture (DMSO/^tBuOH/H₂O) at room temperature for 16 hours. Finally, the oligonucleotides were desalted, analyzed by HPLC and identified by ESI mass spectrometry. Remarkably, the click reaction with azide **11** did not give the desired product. There, the unmodified oligonucleotide strand bearing the alkyne group remained unreacted, which may be due to the steric hindrance by the bulky TBDMS group. On the other hand, the unprotected quinolinol azide **10** gave **DNA 3** and was identified by ESI mass spectrometry as $[M+3H^++Na^+]^{4+}$. Notably, we also observed the oligonucleotide mass with addition of copper as in $[M+2H^++Na^++Cu^+]^{4+}$. However, it is a relevant finding that the click reaction worked well with the quinolinol azide **10**. Though, our observations obtained by mass spectrometry indicate the complexation of a copper ion that is supposed to result from the previous copper catalyzed click reaction. However, to obtain the copper-free oligonucleotide with the alkyne-modified uridine moiety further investigations can be carried out with ion depletion by addition of EDTA.

DNA 3



Scheme 2.6. DNA **3**, bearing a quinolinol modification.

Our preliminary study of the optical properties with the modified oligonucleotide **DNA 3** was carried out with use of HPLC-DAD. There, the purified **DNA 3** displays strong absorption that is predominated by the DNA heterocycles, however, the absorption band at $\lambda_{\text{abs}} = 383$ nm also clearly reveals the presence of the covalently linked quinolinol modification. Interestingly, a significant bathochromic shift is observed when compared with the azido-quinolinol **10** ($\lambda_{\text{abs}} = 339$ nm) as displayed in Figure 2.6, which may be attributed to charge-transfer processes upon copper chelation in **DNA 3**.

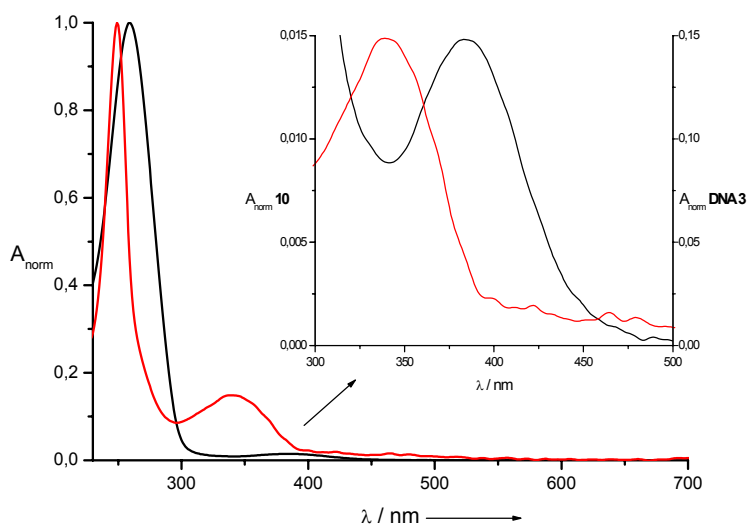


Figure 2.6. Normalized UV/Vis absorption spectra of **10** and purified **DNA 3**. **10** (red): in MeOH (10 μ M); **DNA 3** (black): absorbance of **DNA 3** during HPLC (25.03-25.88 min).

Hence, based on these results quinolinol can be used for prospective studies with complementary oligonucleotide strands, entailing investigations on definite metal complexation in the interior of duplexes as well as metal-mediated triplex formation by conjugation of quinolinol to alkyne-modified DNA bases like EdU. This method will allow a structurally defined arrangement of metal ions along the DNA periphery without interruption of Watson-Crick base pairing through hydrogen bonding.

4.2.3. A new photoswitchable nucleoside bearing a spirobenzopyran

In the following section an efficient and straight-forward synthesis of the photoswitchable nucleoside **22** is presented (Figure 2.7). To avoid long alkyl linkers and to maintain canonical base pairing of 2'-deoxyuridine for further applications,^[1b, 22] the spirobenzopyran modification was attached via a rigid acetylene linker at the C-5 position. Hence, Sonogashira reactions were investigated for the coupling between the alkyne functionalized spirobenzopyran and the halogenated nucleobase or vice versa.

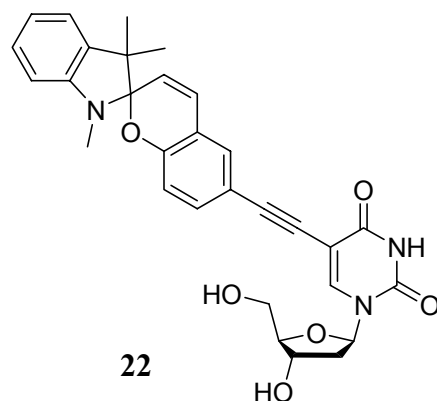
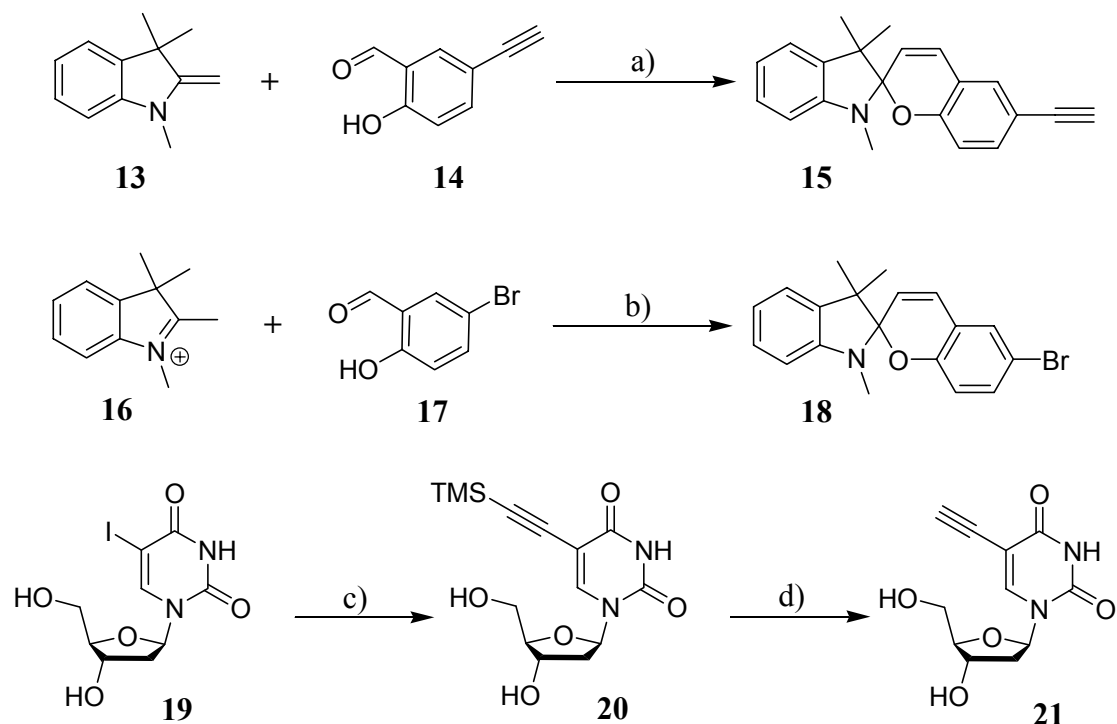


Figure 2.7. Spirobenzopyran nucleoside **22**

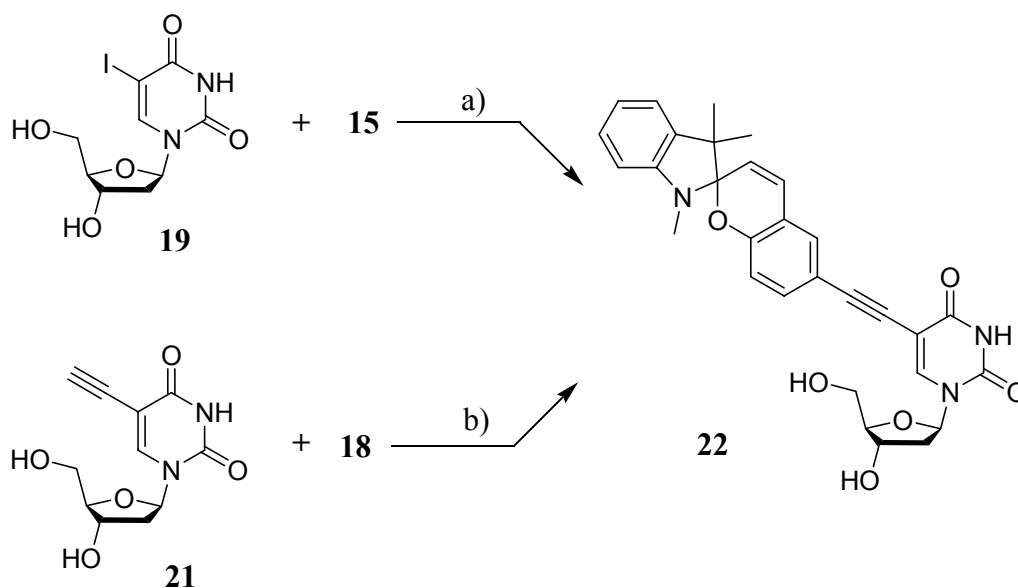
4.2.3.1. Synthesis of spirobenzopyran nucleoside

During the previously described investigation on the synthesis of spirobenzopyrans it was found that under ultrasonic conditions good yields were obtained, accompanied with a significant decrease of the reaction time compared to standard reaction conditions (see Chapter 1). With a reasonable amount of 5-ethynyl-salicylaldehyde **14** in hand, **15** was prepared. The reaction of freshly distilled Fischer base **13** with **14** (1.0 eq) under ultrasonic irradiation conditions in EtOH for 1 hour gave the corresponding N-methyl-spirobenzopyran **15**, which was isolated in 76 % yield as pale blue foam. The 6-bromo compound **18** was prepared from the indolium iodide **16** and **17** with addition of excess triethylamine under ultrasonic conditions in EtOH and obtained as a pale pink solid in assimilable yield (77 %). The synthesis for EdU **21** started from 5-iodo-uridine compound **19** with a Sonogashira cross-coupling reaction with excess of ethynyltrimethylsilane, while $\text{Pd(PPh}_3)_4$ and copper iodide were used as catalysts. The TMS-protected product **20** was obtained in good yield and consequent desilylation went smoothly by using a solution of Bu_4NF in MeOH to provide **21** as colorless foam (82 %).



Scheme 2.7. Synthesis of **15**, **18** and **EdU**. Reagents and conditions: a) EtOH, US, 53 min, 76 %; b) NEt_3 (1.3 eq.), EtOH, US, 2 h, 77 %; c) $(\text{CH}_3)_3\text{SiCCH}$ (9.0 eq.), $\text{Pd(PPh}_3)_4$ (10.5 mol-%), CuI (20.7 mol-%), DMF, NEt_3 , r.t., 4 h, 85 %; d) Bu_4NF (9.7 eq.), MeOH, r.t., 2 d, 82 %.

With a quantity of the spirobenzopyrans **15** and **18** in hand, the Sonogashira cross-coupling was performed subsequently in various ways. First, 5-iodo-2'-deoxyuridine **19** and spirobenzopyran **15** were heated at 55 °C for 3 hours in triethylamine in the presence of $\text{Pd(PPh}_3)_2\text{Cl}_2$ (3 mol-%) and CuI (5 mol-%) as catalysts to provide nucleoside **22** in a yield of 27 %. However, the reaction of **EdU 21** with the spirobenzopyran **18** under similar reaction conditions gave product **22** in 24 %. Interestingly, a better cross-coupling efficiency was observed with increased amounts of catalysts, **15** and 5-iodo-2'-deoxyuridine **19** were dissolved in a mixture of DMF and triethylamine with a relatively high amount of Pd(dppf)Cl_2 (17 mol-%) and CuI (22 mol-%), and the reaction was carried out at room temperature for 26 hours. Thus, the desired nucleoside compound **22** was isolated after column chromatography in 67 % yield.



Scheme 2.8. Synthesis of spirobenzopyran nucleoside **22**. Reagents and conditions: a) **15** (1.2 eq.), Pd(dppf)Cl₂ (17 mol-%), CuI (22 mol-%), DMF, NEt₃, r.t., 26 h, 67 %; b) **18** (1.2 eq.), Pd(PPh₃)₂Cl₂ (4 mol-%), CuI (5 mol-%), 60 °C., 3 h, 24 %.

4.2.3.2. Optical properties of spirobenzopyran nucleoside

The synthesized spirobenzopyran nucleoside **22** was investigated with respect to its photoswitchable properties using UV/Vis spectroscopy. As shown in Figure 2.8, **22** displays photochromic switching properties. After irradiation of the sample with visible light using an amber high-power output LED for 2 minutes, only little absorption in the visible range is detectable. Upon irradiation with UV light ($\lambda = 312$ nm) for 2 minutes an increase in the visible range is observed.

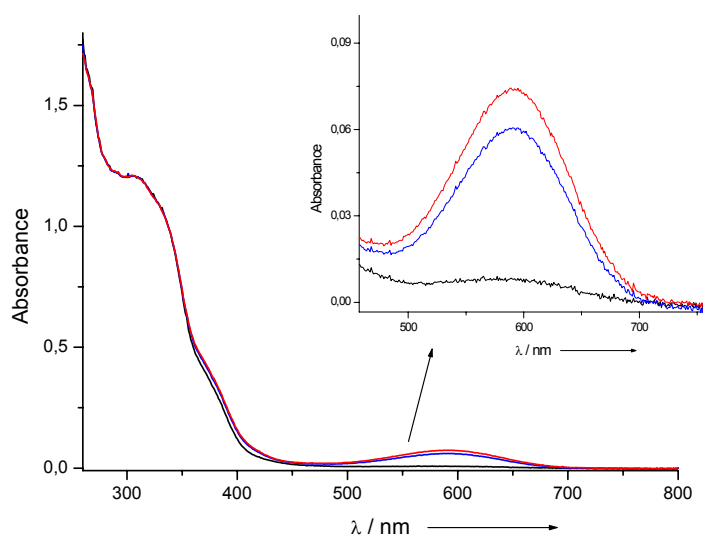


Figure 2.8. UV/Vis absorption spectra of SP- and MC-form interconversion of **22** in MeOH (100 μ M) at r.t.; black: irradiated with Vis, blue: after 2 min irradiation with 312 nm, red: after 3 min irradiation with 312 nm.

The significant formation of the MC-form is also observable by eye, since the former colorless solution turns magenta-red upon UV irradiation. Further irradiation of the sample for 1 minute only leads to little increase, then the absorption maximum ($\lambda_{\text{abs}} = 589$ nm) does not show any further increase, when the irradiation with the UV source is continued. This can be explained as a result of the experimental setup itself, since under the employed irradiation conditions a constant equilibrium between the SP- and the MC-isomers is reached. When this state is established it can be retained by UV irradiation. When the irradiation is abandoned thermal formation of the SP-form is observed.

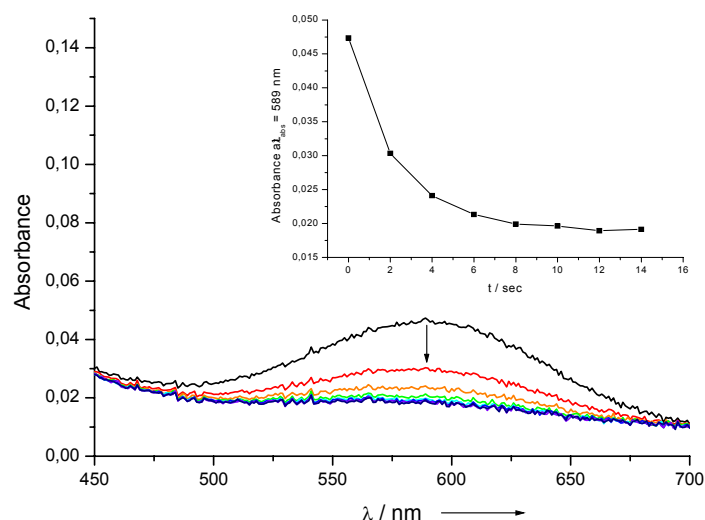


Figure 2.9. UV/Vis absorption spectra of MC- and SP-form thermal interconversion of **22** in MeOH (100 μM) at r.t.; black: after 30 sec irradiation with 312 nm, others: decline followed each 2 sec.

Figure 2.9 displays the thermal interconversion of the nucleoside compound **22**. First, the sample was irradiated with UV light ($\lambda = 312$ nm) for 30 seconds and the UV/Vis spectrum was measured (black spectrum, $\lambda_{\text{abs}} = 589$ nm). The sample was left in the spectrometer and after 2 seconds the next spectrum was recorded (red line). The insert shows the decrease at $\lambda_{\text{abs}} = 589$ nm, which relates to the degeneration of the MC-form and back-formation of the SP-form. Interconversion of the MC-form to the SP-form is even faster when using the Vis high-power output LED, respectively. For further insight of the photoswitching properties of **22**, time-resolved absorption spectroscopy and fluorescence measurements at the photostationary state can be considered.

4.3. Conclusion

In conclusion, the in situ azide formation and click conjugation of fluorescent labels (nile red and perylene) with DNA as an alternative postsynthetic method was described. Using CuAAC, the ethynyl-modified chromophores were conjugated with an azido group that is formed in situ by treatment of presynthesized oligonucleotides incorporating 5-iodo-2'-deoxyuridine with sodium azide on solid support. The method benefits from the advances of solid support synthesis using postsynthetic on bead labeling and completes the repertoire of

click reactions for the modification of DNA by providing a complementary access. In case of the Nile red modified oligonucleotide, comparison of optical properties of the triazolyl and rigid acetylene conjugated DNA revealed remarkably similar optical properties, avoiding decoupling of the chromophore from the DNA base. Additionally, the method can be very useful for nucleic acid chemists since a lot of interesting labels are typically provided with ethynyl groups, with intended use for functional π -systems, and due to commercial accessibility of halogenated precursors as DNA building blocks.

In the present chapter a convenient synthesis of an oligonucleotide containing a quinolinol moiety at a modified uridine was also presented. Therefore, azido-quinolinol derivatives were prepared and incorporated into DNA using off bead labeling by click chemistry. With use of the bioorthogonal click reaction, 5-azido-8-hydroxy-quinolin was successfully conjugated to the oligonucleotide. For further applications, the quinolinol modification can be used for metal-mediated interstrand binding, catalysis and complex, self-assembled supramolecular arrangements of DNA.

Furthermore, an efficient method has been developed for the synthesis of a spirobenzopyran nucleoside, as photoswitchable 2'-deoxyuridine derivative. The method features build up of the spirobenzopyran unit with use of ultrasonic irradiation, followed by Sonogashira cross-coupling reaction with 5-iodo-deoxyuridine to form the new spirobenzopyran nucleoside, respectively. For the employed cross-coupling conditions, the Pd(dppf)Cl₂ catalyst was found superior to the more commonly used Pd(PPh₃)₂Cl₂. The synthesized nucleoside displays reversible switching between its SP- and MC-form by irradiation with UV or Vis light, respectively. The switching process from the MC- to SP-form is also accompanied by thermal interconversion. These optical properties of the nucleoside are of interest for new approaches, e.g. by using reversible photo-modulation on a molecular level in primer extension experiments.

4.4. Experimental Section

General

Conferrable details on reagents, solvents, reaction processing, chromatography, NMR, IR, MS and RP-HPLC are specified in the general section of the experimental part in Chapter 1 and Chapter 3. Compounds **13**, **14** and **16** were prepared according to the synthetic procedures reported in the experimental section in Chapter 1. Compounds **1**, **2** and **TBTA** were prepared

according to the synthetic procedures reported in the experimental section in Chapter 2. Compound **12** was prepared according to the synthetic procedure reported in the experimental section in Chapter 3. 2-cyanoethyl-N,N-diisopropylchlorophosphoramidite **23** was obtained from commercial suppliers. Data on the duplex **DNA 2A** and its respective modified single strand has been published in the literature.^[4a] Unmodified oligonucleotides were purchased from Metabion. Purified water with a resistivity $\geq 18 \text{ M}\Omega \text{ cm}^{-1}$ was used for preparation of buffer solvents, a MARTIN CHRIST Alpha 2-4 freeze dryer was used for lyophilization. Further details on DNA solid support synthesis are listed in Chapter 3 and in the appendix. Synthetic steps involving azides were performed in the dark. Reagents and controlled pore glass (CPG) were purchased from ABI and Glen Research. Mass spectra of the purified oligonucleotides were recorded at the University of Regensburg, Zentrale Analytik Massenspektrometrie, with a ThermoQuest Finnigan TSQ 7000 in negative and positive ionization mode.

UV/Vis and fluorescence spectroscopy

Unless otherwise specified, spectroscopic measurements were performed at 20 °C and quartz glass cuvettes (Starna, 10 mm) were used. UV/Vis spectra were recorded with a Cary BIO 50 and Cary BIO 100 UV/Vis/NIR spectrometer (Varian).

Synthesis of oligonucleotides: On bead coupling protocol and purification

Oligonucleotides were prepared on an Expedite 8909 Synthesizer from Applied Biosystems (ABI) using standard phosphoramidite chemistry on a 1 μmol scale. 5-Iodo-modified uridine was introduced into DNA by using standard coupling conditions. 5-Iodo-modified DNA on CPG was dried under high vacuum after DNA synthesis. 1.0 mL sodium azide (200 mM) solution in DMSO was added to the CPG and heated at 55°C for 1 h, then cooled to r.t. The vial was centrifuged and the supernatant was removed. The CPG was successively washed with DMSO (4 mL) and MeCN (4 mL). The respective ethynyl-modified dyes **1** (or **2**) (300 μL , 50 mM), TBTA (600 μL , 100 mM), $[\text{Cu}(\text{CH}_3\text{CN})_4]\text{PF}_6$ (300 μL , 100 mM) (each in DMSO/tBuOH 3:1) and (+)-sodium L- ascorbate (300 μL , 400 mM in H_2O) were added to the CPG and the mixture was gently shaken for 10 h at r.t. The mixture was centrifuged and the supernatant removed. The CPG was successively washed with DMSO, EtOH and H_2O (each 2 mL) and dried under high vacuum. The oligomers were deprotected and cleaved off the CPG by concentrated NH_4OH solution at r.t. for 18 h. After deprotection and cleavage from

the CPG the oligonucleotides were desalted with use of a prepacked NAP-5 column (GE Healthcare).

The modified oligonucleotides were purified by HPLC on a semipreparative RP-C18 column (300 Å, Supelco) using the following conditions: A) NH_4OAc buffer (50 mM), pH 6.8; B) acetonitrile; gradient 0-30% B over 45 min, flow rate 2.5 mL/min, UV/Vis detection at 260 and 600 nm for **DNA 1**; 260 nm and 450 nm for **DNA 1^{Per}**. The purified **DNA 1** and the purified **DNA 1^{Per}** were identified by ESI-MS.

Optical spectroscopy of oligonucleotides

The oligonucleotides were lyophilized and quantified by their absorbance in 10 mM sodium phosphate buffer at 260 nm on a Varian Cary 100 spectrometer. The oligonucleotides were quantified in 10 mM sodium phosphate buffer by their absorbance at 260 nm using $\epsilon_{260\text{nm}} = 28000 \text{ M}^{-1} \text{ cm}^{-1}$ for Nile red-dU, $\epsilon_{260\text{nm}} = 13800 \text{ M}^{-1} \text{ cm}^{-1}$ for A, $\epsilon_{260\text{nm}} = 10500 \text{ M}^{-1} \text{ cm}^{-1}$ for G, $\epsilon_{260\text{nm}} = 8000 \text{ M}^{-1} \text{ cm}^{-1}$ for T, $\epsilon_{260\text{nm}} = 6500 \text{ M}^{-1} \text{ cm}^{-1}$ for C. $\epsilon_{260\text{nm}}$ of the respective DNA strand: the number of individual bases was multiplied with their respective coefficients and added together. Fluorescence spectra were measured on a Jobin-Yvon Fluoromax 3 fluorimeter with a stepwidth of 1 nm and an integration time of 0.2 s. All spectra were recorded with an excitation and emission bandpass of 5 nm and are corrected for Raman emission from the buffer solution.

Hybridization and melting temperatures

Duplexes were formed by heating of the modified oligonucleotides (2.5 μM) in 10 mM sodium phosphate buffer (pH 7) and 250 mM NaCl in the presence of 1.2 eq. unmodified complementary strand to 90 °C (10 min), followed by slow cooling to r.t. Absorption spectra and melting temperature (2.5 μM DNA, 20-90 °C, 0.7 °C/min, step width 0.5 °C) were recorded on a Varian Cary 100 spectrometer equipped with a 6×6 cell changer unit.

Synthesis of oligonucleotides: Off bead coupling protocol and purification

The 2'-modified uridine **12** was introduced into DNA by using standard coupling conditions, extended coupling time (6.3 min) and increased concentration of 0.1 M (in MeCN). The oligonucleotides were cleaved from the resin and deprotected by treatment with concentrated NH_4OH solution at r.t. for 22 h. The respective azido-modified quinoline compound **10** (or **11**) (300 μL , 50 mM), CuI (300 μL , 100 mM), TBTA (600 μL , 100 mM), each in DMSO/tBuOH (3:1), and sodium ascorbate (300 μL , 400 mM) in H_2O were added to the

oligonucleotide (1 μ mol). The vial was vortexed, shaken 16 h at r.t., and then evaporated to dryness using a SpeedVac. NaOAc (100 μ L, 0.15 mmol) was added, and the mixture was stored for 1 h at r.t. After EtOH precipitation, the oligonucleotides were dissolved in H₂O (500 μ L), desalted, lyophilized and analyzed by RP-HPLC using the following conditions: A) NH₄OAc buffer (50 mM), pH 6.8; B) acetonitrile; gradient 0-30 % B over 45 min, flow rate 1.0 mL/min. **DNA 3** was purified by HPLC on a semipreparative RP-C18 column (300 Å, Supelco) using the following conditions: A) NH₄OAc buffer (50 mM), pH 6.8; B) acetonitrile; gradient 0-30% B over 45 min, flow rate 2.5 mL/min, UV/Vis detection at 260 and 381 nm. The purified **DNA 3** was identified by ESI-MS.

Table 4.1. ESI-MS of modified oligonucleotide single strands (ss).

ss DNA	Sequence	calcd.	found
DNA 1	5'-GCA-GTC-AAX-AAC-ACT-GA-3'	5554.0	1386.6 [M-4H ⁺] ⁴⁺ , 1849.4 [M-3H ⁺] ³⁺ , 1388.6 [M+4H ⁺] ⁴⁺ , 1851.6 [M+3H ⁺] ³⁺
DNA 1 ^{Per}	5'-GCA-GTC-AAX-AAC-ACT-GA-3'	5488.0	1378.7 [M+3H ⁺ +Na ⁺] ⁴⁺ , 1838.2 [M+2H ⁺ +Na ⁺] ³⁺
DNA 3	5'-GCA-GTC-TTX-TTC-ACT-GA-3'	5374.9	1350.0 [M+3H ⁺ +Na ⁺] ⁴⁺ , 1799.7 [M+2H ⁺ +Na ⁺] ³⁺ , 1365.4 [M+2H ⁺ +Na ⁺ +Cu ⁺] ⁴⁺ , 1820.2 [M+H ⁺ +Na ⁺ +Cu ⁺] ³⁺

LUMO calculations

Calculations were performed with Spartan '06 software. Equilibrium geometry calculations were performed using the MMFF for the isolated Nile Red modified 2'-deoxyuridines, then the LUMOs were calculated with the semi-empirical AM1 method and are displayed with IsoValue 0.01.

Fluorescence quantum yields

The fluorescence quantum yields (Φ_{Fl}) were determined by the standard method, taking into account the refractive indices (n) of the solvents:

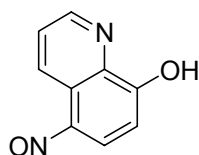
$$\phi_{Fl} = \phi_R \frac{A_R}{A_S} \frac{F_S}{F_R} \frac{n_S^2}{n_R^2}$$

The subscripts “S” and “R” refer to the sample and the reference dye, respectively. *A* is the extinction of the sample solution at the excitation wavelength; *F* is the emission integral over the area of interest. The fluorescence quantum yields were determined by the standard method with Cresyl Violet perchlorate in MeOH as reference ($\Phi_{Fl} = 0.54$).^[30]

Light sources and UV/Vis of spirobenzopyran nucleoside compound **22**

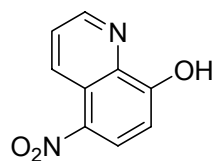
For irradiation experiments a UV hand-held lamp (Herolab, 6 W, $\lambda = 312$ nm) and a Luxeon III Star high-power LED ($\lambda = 590$ nm / amber) were used. UV/Vis spectra of **22** were recorded at r.t. with a Cary BIO 50 UV/Vis/NIR spectrometer (Varian) with scanning speed of 4800 nm/min.

5-nitrosoquinolin-8-ol



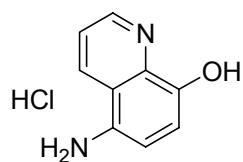
A mixture of 8-quinolinol (10.244 g, 70.57 mmol) in water (93 mL) was cooled in an external ice bath, followed by cautious addition of concentrated H₂SO₄ (4.2 mL). The resulting bright yellow solution was vigorously stirred for 2 hours at room temperature, and an aqueous sodium nitrite solution (10 mL, 74.32 mmol) were added. The viscous yellow reaction mixture was stirred at room temperature for 4 hours. Concentrated sodium hydroxide solution was added periodically to keep the pH of the mixture between 10 and 11 as determined by external damp of universal pH paper. The resulting blood-red solution was then acidified by addition of glacial acetic acid to pH 5 and the precipitate was filtered and dried in vacuo to afford 5-nitrosoquinolin-8-ol as a yellow solid (11.676 g, 95 %)

MS (ESI): *m/z* (%): 175.0 (97) [MH⁺], 216.0 (21) [MH⁺+MeCN], 276.1 (100) [MH⁺+NEt₃]

5-Nitroquinolin-8-ol

To a solution of 5-nitrosoquinolin-8-ol (960 mg, 5.51 mmol) in water (10 mL) there was added finely ground sodium hydroxide (276 mg, 6.90 mmol), followed by hydrogen peroxide (600 μ L, 6.12 mmol). The mixture was stirred at room temperature for 10 minutes, then the temperature was raised to 70 $^{\circ}$ C for 1 hour, slowly cooled to ambient temperature and stirred for 1 hour. Additionally, hydrogen peroxide (200 μ L, 2.04 mmol) was added to complete the conversion. The sodium salt precipitate was removed by filtration and washed with additional water (15 mL). Addition of dilute sulfuric acid afforded 5-nitroquinolin-8-ol as a pale yellow-brown solid (845 mg, 81 %)

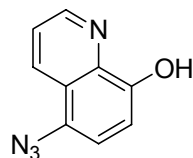
^1H NMR (300 MHz, DMSO- d_6): δ = 12.97 (s, 1 H), 10.66 (s, broad, 1 H), 8.86 (d, 1 H, J = 4.3 Hz), 8.56 (dd, 1 H, J = 1.3, 8.1 Hz), 7.71 (dd, 1 H, J = 4.4, 8.2 Hz), 7.14 (s, 1 H) - MS (ESI): m/z (%): 191.0 (100) [MH^+], 232.0 (53) [$\text{MH}^+ + \text{MeCN}$]

5-aminoquinolin-8-ol hydrochloride

A suspension of 5-nitroquinolin-8-ol (838 mg, 4.41 mmol) in a mixture of MeOH/EtOAc (2.5:1, 35 mL) containing concentrated HCl (1.2 mL) and palladium on activated charcoal (84 mg, 10 % m/m), was stirred under an atmosphere of hydrogen (7 bar) for 26 h. Celite and EDTA were added to the reaction mixture, stirred at room temperature for 1 hour and the solution was filtered to afford an orange brown solid. The residue was recrystallized from EtOH/Et₂O and dried in a desiccator to yield 5-aminoquinolin-8-ol hydrochloride as orange crystals (980 mg, 95 %).

^1H NMR (300 MHz, MeOD): δ = 9.46-8.93 (m, 2 H), 8.05 (s, 1 H), 7.73-7.26 (m, 2 H) - MS (ESI): m/z (%): 161.1 (100) $[\text{MH}^+]$, 202.0 (13) $[\text{MH}^+ + \text{MeCN}]$

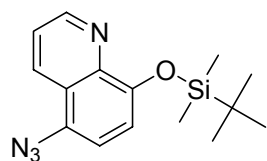
5-azidoquinolin-8-ol



To a solution of 5-aminoquinolin-8-ol hydrochloride (974 mg, 4.09 mmol) in water (5 mL) was added concentrated HCl (0.4 mL) under vigorous stirring. The blood-red solution was cooled to 0 °C and an aqueous solution of sodium nitrite (6.0 mL, 7.74 mmol) was added slowly over 30 minutes. The solution was stirred for 20 minutes at 3 °C, then an aqueous solution of sodium azide (40 mL, 9.71 mmol) was added slowly over 45 minutes. This was stirred for 2 hours at 3 °C, then allowed to slowly warm up to room temperature. After 18 hours the reaction mixture was extracted with Et₂O (4 x 50 mL). The pooled organic layers were washed with water, dried over anhydrous Na₂SO₄ and the solvent was evaporated under reduced pressure to yield 5-azidoquinolin-8-ol as a pale brown solid (228 mg, 30 %).

^1H NMR (300 MHz, CDCl₃): δ = 8.82 (dd, 1 H, J = 1.5, 4.2 Hz), 8.40 (dd, 1 H, J = 1.5, 8.5 Hz), 7.47 (dd, 1 H, J = 4.2, 8.5 Hz), 7.21 (q, 2 H, J = 8.2 Hz) - IR (neat): ν [cm⁻¹] = 3298, 2131, 2112, 1474, 1277 - MS (ESI): m/z (%): 187.1 (100) $[\text{MH}^+]$, 228.1 (18) $[\text{MH}^+ + \text{MeCN}]$

8-[[[(1,1-dimethylethyl)dimethylsilyl]oxy]-5-azidoquinoline

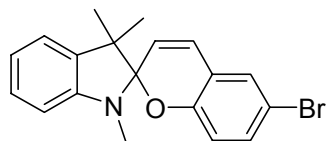


8-quinolionol (80 mg, 0.430 mmol) and imidazole (40 mg, 0.588 mmol) were dissolved in dry CH₂Cl₂ (2.4 mL). Under vigorous stirring, tert-butyldimethylsilyl chloride (80 mg, 0.531

mmol) was added in one portion under argon atmosphere. The solution was stirred in the dark for 69 hours and the solvent was evaporated in vacuo. The remaining solid was purified by gradient flash chromatography on silica gel (Hexane/EtOAc 15:1 to 10:1) to afford 8-[[[(1,1-dimethylethyl)dimethylsilyl]oxy]-5-azidoquinoline as a mucous yellow-orange oil (112 mg, 87 %). $R_f = 0.66$ (Hexane/EtOAc 10:1)

^1H NMR (400 MHz, CDCl_3): $\delta = 8.89$ (dd, 1 H, $J = 1.7, 4.1$ Hz, C2), 8.35 (dd, 1 H, $J = 1.7, 8.5$ Hz, C4), 7.38 (dd, 1 H, $J = 4.1, 8.5$ Hz, C3), 7.19 (d, 1 H, $J = 8.21$, C7), 7.16 (d, 1 H, $J = 8.2$, C6), 1.07 (s, 9 H, 3 x C- CH_3), 0.26 (s, 6 H, 2 x Si- CH_3) - ^{13}C NMR (100 MHz, CDCl_3): $\delta = 150.0$ ($\text{C}_{\text{quat.}}$, C8), 149.4 (CH, C2), 142.2 ($\text{C}_{\text{quat.}}$, C8a), 130.9 (CH, C4), 128.7 ($\text{C}_{\text{quat.}}$, C5), 122.7 ($\text{C}_{\text{quat.}}$, C4a), 121.1 (CH, C3), 117.4 (CH, C6), 114.7 (CH, C7), 25.9 (3 x C- CH_3), 18.8 ($\text{C}_{\text{quat.}}$), -4.0 (2 x Si- CH_3) - MS (ESI): m/z (%): 301.0 (100)[MH^+]

1',3'-dihydro-1',3',3'-trimethyl-6-bromo-spiro[2H-1-benzopyran-2,2'-[2H]indole]

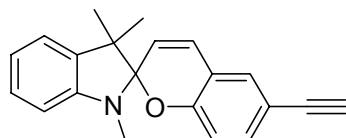


A dry flask was charged with 1,2,3,3-tetramethyl-3H-indolium iodide (335 mg, 1.11 mmol), freshly distilled EtOH (10 mL) and dry NEt_3 (200 μL , 1.43 mmol). 5-bromosalicylaldehyde (220 mg, 1.09 mmol) was added under argon atmosphere and the reaction mixture was sonicated at 35 kHz. The progress of the reaction was monitored by TLC until spot intensity of the product remained constant in successive controls. After 2 hours the solvent was removed under reduced pressure, the residue was dissolved in CH_2Cl_2 and dried over anhydrous Na_2SO_4 . The solution was filtered and the solvent was evaporated under reduced pressure. The residue was dried in vacuo, purified by gradient flash chromatography on silica gel (Hexane/EtOAc 30:1 to 15:1) to yield 1',3'-dihydro-1',3',3'-trimethyl-6-bromo-spiro[2H-1-benzopyran-2,2'-[2H]indole] as pale pink solid (300 mg, 77 %). $R_f = 0.49$ (Hexane/EtOAc 19:1)

^1H NMR (300 MHz, CDCl_3) $\delta = 7.27$ -7.15 (m, 3 H, H-Ar), 7.09 (d, 1 H, $J = 7.2$ Hz, H-Ar), 6.87 (t, 1 H, $J = 7.4$ Hz, H-Ar), 6.80 (d, 1 H, $J = 10.3$ Hz, H-Ar), 6.61 (d, 1 H, $J = 9.2$ Hz, H-

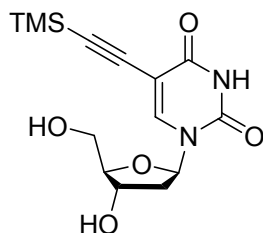
Ar), 6.55 (d, 1 H, $J = 7.7$ Hz, H-Ar), 5.74 (d, 1 H, $J = 10.2$ Hz, H-Ar), 2.74 (s, 3 H, NCH₃), 1.32 (s, 3 H, 3-CH₃), 1.18 (s, 3 H, 3-CH₃) - ¹³C NMR (75 MHz, CDCl₃) $\delta = 153.6$ (C_{quat.}), 148.0 (C_{quat.}), 136.5 (C_{quat.}), 132.2 (+, CH), 129.1 (+, CH), 128.4 (+, CH), 127.7 (+, CH), 121.5 (+, CH), 120.7 (+, CH), 120.6 (C_{quat.}), 119.3 (+, CH), 116.9 (+, CH), 111.8 (C_{quat.}), 106.9 (+, CH), 104.5 (C_{quat.}), 51.9 (C_{quat.}), 28.9 (+, CH₃), 25.9 (+, CH₃), 20.1 (+, CH₃) - MS (EI, 70 eV): m/z (%): 357.0 (100) [M⁺], 339.9 (47) [M⁺-CH₃] - HRMS (EI-MS) calcd. for C₁₉H₁₈BrNO [M⁺]: 355.0572, found: 355.0570

1',3'-dihydro-1',3',3'-trimethyl-6-ethynyl-spiro[2H-1-benzopyran-2,2'-[2H]indole]



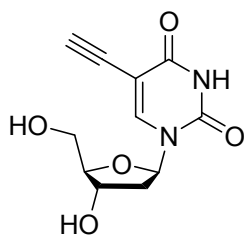
A flask was charged with 5-Ethynyl-2-hydroxybenzaldehyde (490 mg, 3.35 mmol) and dry EtOH (50 mL) and placed in a ultrasonic bath. Freshly distilled 1,3,3-trimethyl-2-methyleneindoline (0.59 mL, 3.33 mmol) was added in one portion under argon atmosphere. The reaction mixture was sonicated at 35 kHz and the progress of the reaction was monitored by TLC until spot intensity of the product remained constant in successive controls. After 53 minutes ultrasonic irradiation was stopped, the solvent was removed under reduced pressure to afford the crude product as blue-green fluffy foam. Purification by gradient flash chromatography on silica gel (Hexane/THF 70:1 to 50:1) afforded 1',3'-dihydro-1',3',3'-trimethyl-6-ethynyl-spiro[2H-1-benzopyran-2,2'-[2H]indole] as pale blue foam (762 mg, 76 %). $R_f = 0.36$ (Hexane/THF 50:1)

¹H NMR (600 MHz, CDCl₃) $\delta = 7.27$ -7.15 (m, 2 H, H-Ar), 7.20 (dt, 1 H, $J = 1.2, 7.7$ Hz, H-Ar), 7.09 (d, 1 H, $J = 7.2$ Hz, H-Ar), 6.87 (t, 1 H, $J = 7.4$ Hz, H-Ar), 6.83 (d, 1 H, $J = 10.3$ Hz, H-Ar), 6.67 (d, 1 H, $J = 8.3$ Hz, H-Ar), 6.55 (d, 1 H, $J = 7.7$ Hz, H-Ar), 5.74 (d, 1 H, $J = 10.2$ Hz, H-Ar), 2.98 (s, 1 H, C \equiv CH), 2.74 (s, 3 H, NCH₃), 1.31 (s, 3 H, CH₃), 1.18 (s, 3 H, CH₃) - ¹³C NMR (150 MHz, CDCl₃) $\delta = 155.0, 148.1, 136.6, 133.7, 130.5, 128.8, 127.6, 121.5, 120.3, 119.3, 118.8, 115.2, 113.5, 106.9, 104.8, 83.6, 75.5, 51.9, 28.9, 25.9, 20.1$ - MS (CI, NH₃): m/z (%): 302.1 (100) [MH⁺] - HRMS (EI-MS) calcd. for C₂₁H₁₉NO [M⁺]: 301.1467, found: 301.1465

5-Trimethylsilylethynyl-2'-deoxyuridine

To a mixture of 5-Iodo-2'-deoxyuridine (512 mg, 1.446 mmol) and dry DMF (10.0 mL) there was added $\text{Pd}(\text{PPh}_3)_4$ (176 mg, 0.152 mmol), CuI (57 mg, 0.299 mmol) and dry NEt_3 (600 μL , 4.304 mmol). The mixture was degassed via freeze-pump-thaw (3 cycles) and Ethynyltrimethylsilane (1.80 mL, 12.99 mmol) was added under nitrogen atmosphere. The reaction mixture was stirred at room temperature for 4 days. The solvent was removed under reduced pressure and the crude was dried in vacuo. Purification by gradient flash chromatography on silica gel ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 200:1 to 10:1) yielded 5-Trimethylsilylethynyl-2'-deoxyuridine as a colorless foam (398 mg, 85 %). $R_f = 0.33$ ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 20:1)

^1H NMR (300 MHz, MeOD): $\delta = 8.33$ (s, 1 H, H-6), 6.23 (t, 1 H, $J = 6.5$ Hz, H-1'), 4.40 (td, 1 H, $J = 3.7, 6.2$ Hz, H-3'), 3.93 (q, 1 H, $J = 3.3$ Hz, H-4'), 3.78 (dq, 2 H, $J = 3.2, 12.0$, H-5'), 2.37-2.16 (m, 2 H, H-2'), 0.20 (s, 9 H, SiMe_3) - ^{13}C NMR (75 MHz, CDCl_3): $\delta = 164.3$ (C-4), 151.2 (C-2), 146.0 (C-6), 100.6 (C-5), 99.1 ($\text{C}_{\text{quat.}}$), 97.4 ($\text{C}_{\text{quat.}}$), 89.2 (C-4'), 87.1 (C-1'), 72.0 (C-3'), 62.6 (C-5'), 41.8 (C-2'), -0.0 (SiMe_3) - MS (ESI): m/z (%): 323.1 (100) $[\text{M}-\text{H}]^+$

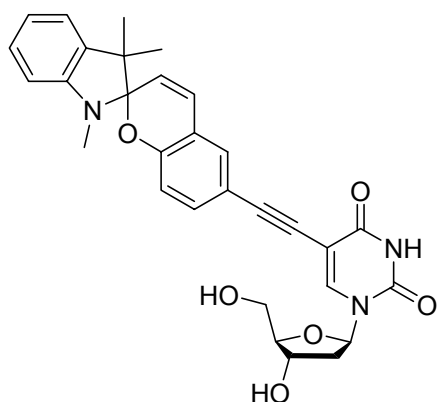
5-ethynyl-2'-deoxyuridine

To a solution of 5-Trimethylsilylethynyl-2'-deoxyuridine (168 mg, 0.519 mmol) in dry MeOH (3 mL) there was slowly added a solution of Bu_4NF (1.588 g, 5.033 mmol) in MeOH

(14 mL) over 32 hours. The mixture was stirred for additional 16 hours at room temperature and the solvent was removed under reduced pressure. Purification by gradient flash chromatography on silica gel ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 20:1 to 10:1) afforded 5-ethynyl-2'-deoxyuridine as a colorless foam (107 mg, 82 %). $R_f = 0.15$ ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 20:1)

^1H NMR (300 MHz, MeOD): $\delta = 8.40$ (s, 1 H, H-6), 6.24 (t, 1 H, $J = 6.6$ Hz, H-1'), 4.40 (m, 1 H, H-3'), 3.94 (q, 1 H, $J = 3.2$ Hz, H-4'), 3.77 (dq, 2 H, $J = 3.2, 12.0$ Hz, H-5'), 2.99 (s, 1 H, $\text{C}\equiv\text{CH}$), 2.36-2.17 (m, 2 H, H-2') - MS (ESI): m/z (%): 250.9 (100) $[\text{M}-\text{H}]^+$

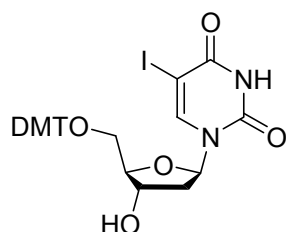
5-[1',3'-dihydro-1',3',3'-trimethyl-spiro[2H-1-benzopyran-2,2'-[2H]indole] 6-ethynyl]-2'-deoxyuridine



A dry flask was charged under nitrogen atmosphere with 1',3'-dihydro-1',3',3'-trimethyl-6-ethynyl-spiro[2H-1-benzopyran-2,2'-[2H]indole] (53 mg, 0.176 mmol), 5-Iodo-2'-deoxyuridine (50 mg, 0.141 mmol), CuI (6 mg, 0.0315 mmol) and [1,1'-Bis(diphenylphosphino)ferrocene]dichloropalladium(II) (20 mg, 0.0245 mmol). Dry DMF (2.0 mL) and dry NEt_3 (100 μL , 0.717 mmol) were added, the mixture was degassed and stirred at ambient temperature for 26 h. Then all was poured into an EtOAc/water mixture (20 mL, v/v 1:1) and the phases separated. The aqueous phase was extracted with CH_2Cl_2 , the pooled organic layers were dried over anhydrous Na_2SO_4 and the solvents were stripped off under reduced pressure. The residue was dried in vacuo and purified by gradient flash chromatography on silica gel ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 10:1 to 5:1) to yield 5-[1',3'-dihydro-1',3',3'-trimethyl-spiro[2H-1-benzopyran-2,2'-[2H]indole] 6-ethynyl]-2'-deoxyuridine as glistening green crystals (50 mg, 67 %). $R_f = 0.29$ ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 10:1)

^1H NMR (600 MHz, MeOD): δ = 8.36 (s, 1 H, H-6), 7.27 (d, 1 H, J = 2.0 Hz), 7.23 (dd, 1 H, J = 8.4 Hz), 7.10 (dt, 1 H, J = 7.6 Hz), 7.03 (d, 1 H, J = 7.2 Hz), 6.92 (d, 1 H, J = 10.3 Hz), 6.78 (t, 1 H, J = 7.1 Hz), 6.61 (d, 1 H, J = 8.4 Hz), 6.52 (d, 1 H, J = 7.8 Hz), 6.26 (t, 1 H, J = 6.6 Hz, H-1'), 5.80 (d, 1 H, J = 10.3 Hz), 4.43-4.40 (m, 1 H, H-3'), 3.94 (q, 1 H, J = 3.3 Hz, H-4'), 3.83 (dd, 1 H, J = 3.0, 12.0 Hz, H-5'), 3.75 (dd, 1 H, J = 3.4, 12.0 Hz, H-5'), 2.70 (s, 3 H, N-CH₃), 2.34-2.29 (m, 1 H, H-2'), 2.28-2.22 (m, 1 H, H-2'), 1.26 (s, 3 H, CH₃), 1.14 (s, 3 H, CH₃) - ^{13}C NMR (150 MHz, MeOD): δ = 164.4, 156.2, 151.2, 149.5, 144.5 (C6), 137.8, 134.2, 131.1, 130.0, 128.6, 122.4, 121.4, 120.5, 120.4, 116.0, 107.9, 106.3, 100.9, 93.9, 89.1 (C-4'), 87.0 (C-1'), 80.1, 72.0 (C-3'), 62.6 (C-5'), 52.9, 41.8 (C-2'), 29.2 (NCH₃), 26.3 (CH₃), 20.4 (CH₃), 9.3 - MS (ESI): m/z (%): 528.2 (100) [MH^+]

5-Iodo-5'-O-(4,4'-dimethoxytrityl)-2'-deoxyuridine

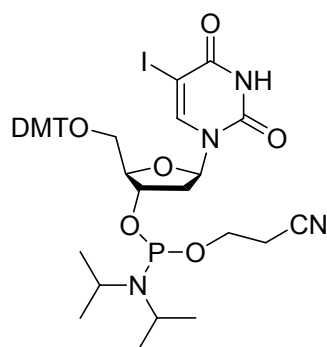


5-Iodo-2'-deoxyuridine (1.08 g, 3.05 mmol) was dried by co-evaporation from dry pyridine (2 x 30 mL), then dry pyridine (40 mL), 4,4'-dimethoxytriphenylmethyl chloride (1.249 g, 3.69 mmol) and dry NEt₃ (1.3 mL, 9.33 mmol) were added. The reaction mixture was stirred for 24 hours at room temperature in the dark and MeOH (8 mL) were added. Following, the mixture was evaporated to dryness and repeated co-evaporation with toluene removed final traces of pyridine to yield yellow foam. The crude product was dissolved in CH₂Cl₂ and washed with aqueous NaHCO₃ and dried over anhydrous MgSO₄. The solvent was evaporated under reduced pressure, the residue was dried in vacuo and the crude product was purified by gradient flash chromatography on silica gel (CH₂Cl₂/MeOH 100:0 + 1 % NEt₃ to 15:1 + 1 % NEt₃) to yield 5-Iodo-5'-O-(4,4'-dimethoxytrityl)-2'-deoxyuridine as glistening white foam (1.885 g, 94 %). R_f = 0.41 (CH₂Cl₂/MeOH 20:1 + 1 % NEt₃)

^1H NMR (300 MHz, CDCl₃): δ = 8.13 (s, 1 H, H-6), 7.44-7.37 (m, 2 H, DMT), 7.35-7.18 (m, 7 H, DMT), 6.83 (d, 4 H, J = 8.8 Hz, DMT), 6.32 (dd, 1 H, J = 5.7, 7.9 Hz, H-1'), 4.57 (m, 1 H, H-3'), 4.12 (d, 1 H, J = 2.5 Hz, H-4'), 3.77 (s, 6 H, 2 x OCH₃), 3.35 (d, 2 H, J = 3.0 Hz, H-

5'), 2.51 (ddd, 1 H, $J = 2.3, 5.6, 13.4$ Hz, H-2'), 2.26 (ddd, 1 H, $J = 5.9, 7.9, 13.6$ Hz, H-2') - ^{13}C NMR (100 MHz, CDCl_3): $\delta = 162.0, 158.6, 151.4, 144.0, 135.6, 135.5, 130.1, 130.1, 128.1, 128.0, 127.0, 113.4, 86.9, 86.3, 85.4, 72.1, 69.6, 63.7, 55.3, 41.5$ - MS (ESI): m/z (%): 655.2 (100) $[\text{M-H}]^+$

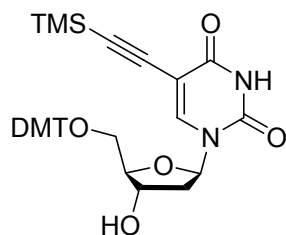
5-Iodo-5'-O-(4,4'-dimethoxytrityl)-2'-deoxyuridine-3'-(2-cyanoethyl-N,N-diisopropylphosphoramidite)



A dry flask was purged with argon, charged with 5-Iodo-5'-O-(4,4'-dimethoxytrityl)-2'-deoxyuridine (217 mg, 0.331 mmol) and dry CH_2Cl_2 (13.0 mL), and degassed. The mixture was cooled on ice, anhydrous NEt_3 (650 μL , 4.664 mmol) and 2-cyanoethyl-N,N-Diisopropylchlorophosphoramidite (240 μL , 1.076 mmol) were added under argon atmosphere and the solution was stirred at room temperature for 1 hour. Following, the reaction mixture was poured into aqueous saturated NaHCO_3 solution (30 mL), additional CH_2Cl_2 (20 mL) was added and the layers separated. The organic layer was washed with brine, dried over anhydrous Na_2SO_4 and the solvent was evaporated under reduced pressure. The remaining ivory solid was purified by flash chromatography on silica gel ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 100:1 + 1 % DIPEA to 50:1 + 1 % DIPEA). After lyophilisation from MeCN, 5-Iodo-5'-O-(4,4'-dimethoxytrityl)-2'-deoxyuridine-3'-(2-cyanoethyl-N,N-diisopropylphosphoramidite) was obtained as white solid (260 mg, 92 %). $R_f = 0.34$ ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 100:1 + 1 % DIPEA)

Analytical data is reported in the literature.^[31]

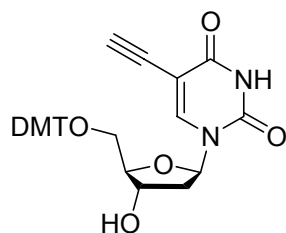
5-Trimethylsilylethynyl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxyuridine



5-Iodo-5'-O-(4,4'-dimethoxytrityl)-2'-deoxyuridine (1.022 g, 1.557 mmol) was dissolved in dry tetrahydrofuran (8.5 mL). Under nitrogen atmosphere, [1,1'-Bis(diphenylphosphino)-ferrocene]dichloropalladium(II) (161 mg, 0.197 mmol), CuI (35 mg, 0.184 mmol) and dry piperidine (2.5 mL) were added and the solution was degassed. Under a flow of nitrogen, Ethynyltrimethylsilane (440 μ L, 3.176 mmol) was added and the reaction mixture was stirred at room temperature for 19 hours. A saturated aqueous NH_4Cl solution (15 mL) was added and the mixture was extracted with EtOAc (2 x 25 mL). The organic layers were pooled and dried over anhydrous Na_2SO_4 . The solvent was evaporated and the remaining crude product was purified by gradient flash chromatography on silica gel ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 20:1 + 1 % NEt_3 to 5:1 + 1 % NEt_3) to yield 5-Trimethylsilylethynyl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxyuridine as ivory foam (773 mg, 79 %). R_f = 0.05 ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 20:1 + 1 % NEt_3)

^1H NMR (400 MHz, CDCl_3): δ = 7.99 (s, 1 H, H-6), 7.46-7.41 (m, 2 H, DMT), 7.37-7.17 (m, 7 H, DMT), 6.88-6.80 (m, 4 H, DMT), 6.27 (dd, 1 H, J = 5.8, 7.6 Hz, H-1'), 4.44 (m, 1 H, H-3'), 4.07 (dd, 1 H, J = 3.3, 6.2 Hz, H-4'), 3.78 (s, 6 H, 2 x OCH_3), 3.41 (dd, 1 H, J = 3.5, 10.6 Hz, H-5'), 3.31 (dd, 1 H, J = 3.8, 10.6 Hz, H-5'), 2.47 (ddd, 1 H, J = 2.5, 5.6, 13.5 Hz, H-2'), 2.20 (ddd, 1 H, J = 6.5, 7.6, 13.6 Hz, H-2'), 0.01 (s, 9 H, 3 x CH_3) - ^{13}C NMR (100 MHz, CDCl_3): δ = 161.4 ($\text{C}_{\text{quat.}}$), 158.6 ($\text{C}_{\text{quat.}}$), 149.3 ($\text{C}_{\text{quat.}}$), 144.4 ($\text{C}_{\text{quat.}}$), 142.6 (CH, C-6), 135.6 ($\text{C}_{\text{quat.}}$), 130.0 (CH), 128.0 (CH), 127.9 (CH), 126.9 (CH), 113.3 (CH), 100.5 ($\text{C}_{\text{quat.}}$), 99.6 ($\text{C}_{\text{quat.}}$), 94.9 ($\text{C}_{\text{quat.}}$), 86.4 (CH, C-4'), 85.7 (CH, C-1'), 72.3 (CH, C-3'), 63.5 (CH_2 , C-5'), 55.2 (OCH_3), 41.4 (CH_2 , C-2'), -0.4 (CH_3 , SiMe_3) - MS (ESI): m/z (%): 625.3 (100) [M-H^+]

5-Ethynyl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxyuridine



Route A:

To a solution of 5-Trimethylsilylethynyl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxyuridine (551 mg, 0.88 mmol) in dry THF (11 mL), Bu₄NF (596 mg, 1.89 mmol) was added. The reaction mixture was stirred at room temperature for 24 hours. Water was added and the mixture was extracted with CH₂Cl₂ (2 x 80 mL). The pooled organic layers were concentrated in vacuo, EtOAc and brine were added. Further extraction with EtOAc gave a colorless layer. Drying over anhydrous Na₂SO₄ and evaporation of the solvent under reduced pressure gave 5-ethynyl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxyuridine as colorless foam (473 mg, 97 %).

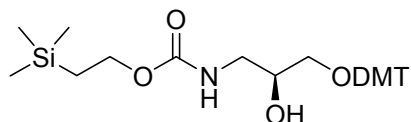
Route B:

A flask was charged with 5-ethynyl-2'-deoxyuridine (158 mg, 0.63 mmol), dry pyridine (7.0 mL), dry NEt₃ (100 μL, 0.717 mmol) and 4-DMAP (8 mg, 0.0655 mmol). The mixture was degassed, 4,4'-dimethoxytriphenylmethyl chloride (247 mg, 0.729 mmol) was added in one portion and the reaction mixture was stirred at room temperature for 20 hours. Following, the solvent was removed under reduced pressure and the crude product was purified by gradient flash chromatography on silica gel (CH₂Cl₂/MeOH 30:1 + 1 % NEt₃ to 10:1 + 1 % NEt₃) to afford 5-ethynyl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxyuridine as a colorless foam (320 mg, 92 %). R_f = 0.28 (CH₂Cl₂/MeOH 50:1 + 1 % NEt₃)

¹H NMR (600 MHz, CDCl₃): δ = 8.09 (s, 1 H, H-6), 7.40 (d, 2 H, J = 7.4 Hz, DMT), 7.31 (dd, 4 H, J = 8.8 Hz, DMT), 7.27-7.24 (m, 2 H, DMT), 7.17 (t, 1 H, J = 7.3 Hz, DMT), 6.81 (dd, 4 H, J = 9.0 Hz), 6.29 (dd, 1 H, J = 5.9, 7.5 Hz, H-1'), 4.61 (td, 1 H, J = 2.6, 5.5 Hz, H-3'), 4.15 (dd, 1 H, J = 3.0, 5.9 Hz, H-4'), 3.76 (s, 6 H, 2 x OCH₃), 3.38 (dd, 1 H, J = 3.7, 10.7 Hz, H-5'), 3.29-3.26 (m, 1 H, H-5'), 2.83 (s, 1 H, C≡CH), 2.57 (ddd, 1 H, J = 2.7, 5.8, 13.4 Hz, H-2'), 2.25 (ddd, 1 H, J = 5.9, 7.5, 13.5 Hz, H-2') - ¹³C NMR (150 MHz, CDCl₃): δ = 161.5, 158.5, 158.5, 155.2, 149.2, 146.0, 144.5, 143.9 (CH, C6), 135.6, 135.4, 130.0, 130.0, 128.0, 127.9, 126.8, 113.2, 113.2, 98.9, 86.9, 86.6 (C-4'), 85.8 (C-1'), 81.6 (C≡CH), 71.8 (C-3') 55.2

(2 x OCH₃), 41.6 (C-2'), 39.3, 24.0, 19.7, 13.6, 8.8 - MS (ESI): m/z (%): 302.9 (100) [DMT⁺], 572.1 (25) [M+NH₄⁺]

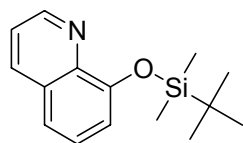
2-(trimethylsilyl)ethyl-(S)-3-(bis(4-methoxyphenyl)(phenyl)methoxy)-2-hydroxypropylcarbamate



To a solution of (S)-1-amino-3-(bis(4-methoxyphenyl)(phenyl)methoxy)propan-2-ol (993 mg, 2.09 mmol) in dry CH₂Cl₂ (107 mL), dry N,N-diisopropylethylamine (4.4 mL, 25.77 mmol) and 4-nitrophenyl-2-(trimethylsilyl)ethyl carbonate (1.836 g, 6.48 mmol) were added. The solution was stirred at room temperature in the dark for 75 hours. The solvents were removed under reduced pressure to yield the crude product as viscous yellow oil that was purified by gradient flash chromatography on silica gel (Hexane/EtOAc 4:1 + 1 % DIPEA to 1:1 + 1 % DIPEA) to give 2-(trimethylsilyl)ethyl-(S)-3-(bis(4-methoxyphenyl)-(phenyl)methoxy)-2-hydroxypropylcarbamate as a glistening pale yellow foam (2.076 g, 60 %). R_f = 0.32 (Hexane/EtOAc 2:1 + 1 % DIPEA)

¹H NMR (300 MHz, CDCl₃) 7.43-7.33 (m, 2 H, arom.), 7.30-7.22 (m, 7 H, arom.), 6.84-6.77 (m, 4 H, arom.), 4.15-4.06 (m, 2 H), 3.90-3.80 (m, 1 H), 3.76 (s, 6 H, 2 x OCH₃), 3.58-3.23 (m, 2 H), 3.18-3.11 (m, 2 H), 0.99-0.90 (m, 2 H), 0.00 (s, 9 H, Si(CH₃)₃) - ¹³C NMR (100 MHz, MeOD) 160.1, 159.3, 146.5, 137.42, 137.40, 131.3, 129.4, 128.7, 127.7, 114.0, 87.4, 71.1, 66.9, 63.9, 55.7 (+, CH₃, 2 x OCH₃), 45.3, 18.7, 14.5, -1.4 (+, CH₃, Si(CH₃)₃) - MS (ESI, CH₂Cl₂/MeOH + 10 mM NH₄OAc): m/z (%): 303.0 (100) [DMT⁺], 555.3 (36) [M+NH₄⁺]

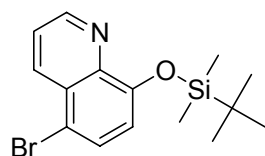
8-[[[(1,1-dimethylethyl)dimethylsilyl]oxy]-quinoline



To a solution of 8-quinolinol (656 mg, 4.52 mmol) in dry CH_2Cl_2 (9 mL), imidazole (334 mg, 4.91 mmol) and tert-butyldimethylsilyl chloride (770 mg, 5.11 mmol) were added. The reaction was stirred at room temperature for 24 hours. Following, the reaction mixture was filtered and the solvent was removed under reduced pressure. Purification by flash chromatography on silica gel (Hexane/EtOAc 95:5) afforded 8-[[[(1,1-dimethylethyl)dimethylsilyl]oxy]-quinoline as colorless oil (983 mg, 84 %). $R_f = 0.33$

^1H NMR (600 MHz, CDCl_3): $\delta = 8.88$ (dd, 1 H, $J = 1.7, 4.1$ Hz, C2), 8.11 (dd, 1 H, $J = 1.7$ Hz, $J = 8.3$ Hz, C4), 7.44-7.41 (m, 2 H, C5/7), 7.37 (dd, 1 H, $J = 4.1, 8.3$ Hz, C3), 7.21 (dd, 1 H, $J = 3.8, 5.0$ Hz, C6), 1.11 (s, 9 H, 3 x C- CH_3), 0.31 (s, 6 H, 2 x Si- CH_3) - ^{13}C NMR (150 MHz, CDCl_3): $\delta = 152.9$ ($\text{C}_{\text{quat.}}$, C8), 148.6 (CH, C2), 142.2 ($\text{C}_{\text{quat.}}$, C4a), 135.7 (CH, C4), 129.7 ($\text{C}_{\text{quat.}}$, C8a), 126.9 (CH, C7), 121.2 (CH, C3), 120.4 (CH, C5), 117.9 (CH, C6), 26.0 (3 x C- CH_3), 18.9 ($\text{C}_{\text{quat.}}$), -3.9 (2 x Si- CH_3) - MS (CI, NH_3): m/z (%): 260.1 (100) [MH^+]

8-[[[(1,1-dimethylethyl)dimethylsilyl]oxy]-5-bromoquinoline

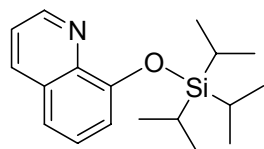


8-[[[(1,1-dimethylethyl)dimethylsilyl]oxy]-quinoline (102 mg, 0.394 mmol) was dissolved in dry CH_2Cl_2 (2 mL) and cooled to -15°C . Under vigorous stirring, 2,4,4,6-tetrabromo-2,5-cyclohexadienone (161 mg, 0.393 mmol) was added in small portions over 1 hour. The solution was allowed to slowly warm to ambient temperature and stirred for 3 hours. The solvent was removed under reduced pressure to yield the crude product as an orange-brown oil. After purification by flash chromatography on silica gel (toluene) 8-[[[(1,1-

dimethylethyl)dimethylsilyl]oxy]-5-bromoquinoline was obtained as lustrous orange crystals (95 mg, 72 %). $R_f = 0.58$

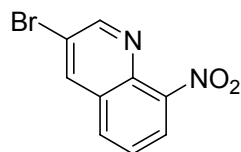
^1H NMR (600 MHz, CDCl_3): $\delta = 8.87$ (dd, 1 H, $J = 1.6, 4.1$ Hz, C2), 8.45 (dd, 1 H, $J = 1.6, 8.6$ Hz, C4), 7.68 (d, 1 H, $J = 8.2$ Hz, C6), 7.47 (dd, 1 H, $J = 4.1, 8.6$ Hz, C3), 7.07 (d, 1 H, $J = 8.2$ Hz, C7), 1.09 (s, 9 H, 3x C- CH_3), 0.29 (s, 6 H, 2 x Si- CH_3) - ^{13}C NMR (150 MHz, CDCl_3): $\delta = 152.9$ ($\text{C}_{\text{quat.}}$, C8), 149.0 (CH, C2), 142.9 ($\text{C}_{\text{quat.}}$, C8a), 135.3 (CH, C4), 130.5 (CH, C6), 128.5 ($\text{C}_{\text{quat.}}$, C4a), 122.3 (CH, C3), 118.3 (CH, C7), 112.3 ($\text{C}_{\text{quat.}}$, C5), 25.9 (3 x C- CH_3), 18.9 ($\text{C}_{\text{quat.}}$), -3.9 (2 x Si- CH_3) - MS (CI, NH_3): m/z (%): 338.0 (100) [MH^+]

8-[[triisopropylsilyl]oxy]-quinoline



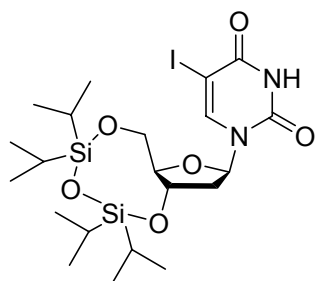
To a solution of 8-quinolinol (355 mg, 2.45 mmol) in dry DMF (7 mL), imidazole (183 mg, 2.69 mmol) and triisopropylsilyl chloride (570 μL , 2.69 mmol) were added. The pale yellow reaction mixture was stirred at room temperature for 20 hours. The solvent was removed under reduced pressure and the yellow mucous residue was purified by flash chromatography on silica gel (Hexane/EtOAc 95:5) to yield 8-[[triisopropylsilyl]oxy]-quinoline as colorless oil (602 mg, 82 %). $R_f = 0.22$

^1H NMR (300 MHz, CDCl_3): $\delta = 8.85$ (dd, 1 H, $J = 1.7, 4.1$ Hz), 8.09 (dd, 1 H, $J = 1.8, 8.3$ Hz), 7.40-7.33 (m, 3 H), 7.18 (dd, 1 H, $J = 3.6, 5.3$ Hz), 1.43 (td, 1 H, $J = 7.5, 14.9$ Hz), 1.13 (d, 1 H, $J = 7.4$ Hz)

3-bromo-8-nitroquinoline

A solution of 8-nitroquinolin (305 mg, 1.75 mmol) in glacial acetic acid (4 mL) was heated at 110 °C. Following, N-bromosuccinimide (343 mg, 1.93 mmol) was added over one hour, the reaction mixture heated at 125 °C for 5 minutes and then allowed to cool to room temperature over 2 hours. After cooling, water (45 mL) was added. This was extracted with CH₂Cl₂ (2 x 40 mL), and the pooled extracts stripped off solvent under reduced pressure. The resulting pale yellow solid was purified by flash chromatography on silica gel (Hexane/EtOAc 5:1) to afford 3-bromo-8-nitroquinoline as an ivory-white solid (326 mg, 74 %). $R_f = 0.32$

¹H NMR (600 MHz, CDCl₃): δ = 9.01 (d, 1 H, J = 2.3 Hz, C2), 8.39 (dd, 1 H, J = 0.4, 2.3 Hz, C4), 8.02 (dd, 1 H, J = 1.4, 7.6 Hz, C7), 7.93 (ddd, 1 H, J = 0.4, 1.4, 8.3 Hz, C5), 7.62 (dd, 1 H, J = 7.5, 8.3 Hz, C6) - ¹³C NMR (150 MHz, CDCl₃): δ = 153.7 (CH, C2), 148.4 (C_{quat.}, C8), 137.6 (C_{quat.}, C8a), 137.3 (CH, C4), 131.0 (CH, C5), 129.8 (C_{quat.}, C4a), 126.7 (CH, C6), 124.0 (CH, C7), 119.3 (C_{quat.}, C3) - MS (EI, 70 eV): m/z (%): 127.2 (100) [M^+ -NO₂-Br], 252.0 (59) [M^+]

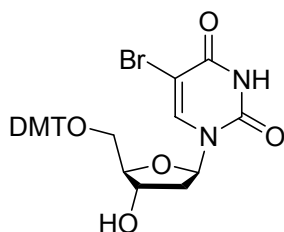
5-Iodo-5'-O-(tetraisopropylidisiloxane-1,3-diyl)-2'-deoxyuridine

To a dry flask containing 5-iodo-2'-deoxyuridine (998 mg, 2.82 mmol) in dry pyridine (20 mL) and 1,3-dichloro-1,1,3,3-tetraisopropylidisiloxane (980 μ L, 3.14 mmol) were added. The reaction mixture was stirred in the dark at room temperature for 7 hours. Pyridine was removed under reduced pressure and the residue was dried in vacuo to constant weight. The

remaining solid was purified by gradient flash chromatography on silica gel (CH₂Cl₂/MeOH 50:1 to 10:1) to afford 5-Iodo-5'-O-(tetraisopropylidisiloxane-1,3-diyl)-2'-deoxyuridine as a colorless foam (1.620 g, 96 %). *R*_f = 0.68 (CH₂Cl₂/MeOH 10:1)

¹H NMR (600 MHz, CDCl₃): δ = 9.57 (s, 1 H, NH), 8.01 (s, 1 H, H-6), 6.00 (dd, 1 H, *J* = 1.9, 7.2 Hz, H-1'), 4.45 (ddd, 1 H, *J* = 7.3, 8.1, 10.1 Hz, H-3'), 4.13 (dd, 1 H, *J* = 2.4, 13.2 Hz, H-5'), 4.01 (dd, 1 H, *J* = 3.0, 13.2 Hz, H-5'), 3.77 (ddd, 1 H, *J* = 2.4, 3.0, 8.1 Hz, H-4'), 2.50 (ddd, 1 H, *J* = 7.2, 10.1, 13.6 Hz, H-2'), 2.27 (ddd, 1 H, *J* = 1.9, 7.3, 13.6 Hz, H-2'), 1.11 (m, 28 H, 4 x ⁱPr) - ¹³C NMR (150 MHz, CDCl₃): δ = 160.2, 149.4, 144.0, 85.3, 84.6, 68.1, 67.1, 59.9, 39.9, 17.7-16.8 (8 x ⁱPr, CH₃), 13.5-12.4 (4 x ⁱPr, CH) - MS (ESI): *m/z* (%): 597.1 (100) [MH⁺], 614.1 (24) [M+NH₄⁺], 1193.4 (11) [2MH⁺], 1210.4 (19) [2M+NH₄⁺],

5-Bromo-5'-O-(4,4'-dimethoxytrityl)-2'-deoxyuridine



To a solution of 5-bromo-2'-deoxyuridine (368 mg, 1.198 mmol) in dry pyridine (16 mL), 4,4'-dimethoxytriphenylmethyl chloride (493 mg, 1.455 mmol) and dry NEt₃ (550 μL, 3.946 mmol) were added. The reaction mixture was stirred for 21 hours at room temperature. The reaction mixture was evaporated to dryness and repeated co-evaporation with toluene removed final traces of pyridine to afford orange oil. Following, the crude product was dissolved in a mixture of CH₂Cl₂ and water. The aqueous layer was extracted with CH₂Cl₂ (2 x) and the pooled organic layers were dried over anhydrous MgSO₄. The solvent was evaporated under reduced pressure and the crude product was purified by gradient flash chromatography on silica gel (CH₂Cl₂/MeOH 100:0 + 1 % NEt₃ to 15:1 + 1 % NEt₃) to yield 5-Bromo-5'-O-(4,4'-dimethoxytrityl)-2'-deoxyuridine as glistening white foam (664 mg, 91 %). *R*_f = 0.20 (CH₂Cl₂/MeOH 20:1 + 1 % NEt₃)

¹H NMR (300 MHz, CDCl₃): δ = 8.04 (s, 1 H, H-6), 7.40 (d, 2 H, *J* = 7.3 Hz, DMT), 7.35-7.17 (m, 7 H, DMT), 6.83 (d, 4 H, *J* = 8.6 Hz, DMT), 6.33 (dd, 1 H, *J* = 5.8, 7.6 Hz, H-1'),

4.57 (m, 1 H, H-3'), 4.10 (d, 1 H, $J = 2.7$ Hz, H-4'), 3.78 (s, 6 H, 2 x OCH₃), 3.37 (m, 2 H, H-5'), 2.51 (ddd, 1 H, $J = 2.6, 5.7, 13.5$ Hz, H-2'), 2.27 (ddd, 1 H, $J = 6.0, 7.6, 13.6$ Hz, H-2')

4.5. References

- [1] a)K. Burgess, D. Cook, *Chem. Rev.* **2000**, *100*, 2047; b)C. Wanninger-Weiß, F. Di Pasquale, T. Ehrenschwender, A. Marx, H.-A. Wagenknecht, *Chem. Commun.* **2008**, 1443; c)M. Hocek, M. Fojta, *Org. Biomol. Chem.* **2008**, *6*, 2233.
- [2] a)S. L. Beaucage, M. H. Caruthers, *Tetrahedron Lett.* **1981**, *22*, 1859; b)M. H. Caruthers, *Acc. Chem. Res.* **1991**, *24*, 278; c)M. Gilar, E. S. P. Bouvier, *J. Chromatogr. A* **2000**, *890*, 167.
- [3] S. H. Weisbrod, A. Marx, *Chem. Commun.* **2008**, 5675.
- [4] a)R. Varghese, H.-A. Wagenknecht, *Chem. Eur. J.* **2009**, *15*, 9307; b)R. Varghese, H.-A. Wagenknecht, *Org. Biomol. Chem.* **2010**, *8*, 526.
- [5] M. Rist, N. Amann, H.-A. Wagenknecht, *Eur. J. Org. Chem.* **2003**, *13*, 2498.
- [6] P. M. E. Gramlich, S. Warncke, J. Gierlich, T. Carell, *Angew. Chem., Int. Ed.* **2008**, *47*, 3442.
- [7] T. L. Hill, J. W. Mayhew, *J. Chromatogr. A* **1990**, *512*, 415.
- [8] V. V. Rostovtsev, L. G. Green, V. V. Fokin, K. B. Sharpless, *Angew. Chem., Int. Ed.* **2002**, *41*, 2596.
- [9] Q. Wang, T. R. Chan, R. Hilgraf, V. V. Fokin, K. B. Sharpless, M. G. Finn, *J. Am. Chem. Soc.* **2003**, *125*, 3192.
- [10] a)J. Gierlich, G. A. Burley, P. M. E. Gramlich, D. M. Hammond, T. Carell, *Org. Lett.* **2006**, *8*, 3639; b)S. Berndt, N. Herzig, P. Kele, D. Lachmann, X. Li, O. S. Wolfbeis, H.-A. Wagenknecht, *Bioconjugate Chem.* **2009**, *20*, 558.
- [11] P. Kocalka, A. H. El-Sagheer, T. Brown, *ChemBioChem* **2008**, *9*, 1280.
- [12] R. Kumar, A. El-Sagheer, J. Tumpene, P. Lincoln, L. M. Wilhelmsson, T. Brown, *J. Am. Chem. Soc.* **2007**, *129*, 6859.
- [13] M. F. Jacobsen, J. B. Ravensbaek, K. V. Gothelf, *Org. Biomol. Chem.* **2010**, *8*, 50.
- [14] G. P. Miller, E. T. Kool, *J. Org. Chem.* **2004**, *69*, 2404.
- [15] I. Géci, V. V. Filichev, E. B. Pedersen, *Chem. Eur. J.* **2007**, *13*, 6379.
- [16] C. Bouillon, A. Meyer, S. Vidal, A. Jochum, Y. Chevolot, J.-P. Cloarec, J.-P. Praly, J.-J. Vasseur, F. Morvan, *J. Org. Chem.* **2006**, *71*, 4700.

- [17] J. Lietard, A. Meyer, J.-J. Vasseur, F. Morvan, *J. Org. Chem.* **2008**, 73, 191.
- [18] V. R. Sirivolu, P. Chittepu, F. Seela, *ChemBioChem* **2008**, 9, 2305.
- [19] A. Salic, T. J. Mitchison, *Proc. Natl. Acad. Sci. U. S. A.* **2008**, 105, 2415.
- [20] a) S. Gourdain, A. Martinez, C. Petermann, D. Harakat, P. Clivio, *J. Org. Chem.* **2009**, 74, 6885; b) R. K. Evans, B. E. Haley, *Biochemistry* **1987**, 26, 269.
- [21] D. K. Lee, R. K. Evans, J. Blanco, J. Gottesfeld, J. D. Johnson, *J. Biol. Chem.* **1991**, 266, 16478.
- [22] R. Varghese, P. K. Gajula, T. K. Chakraborty, H.-A. Wagenknecht, *Synlett* **2009**, 20, 3252.
- [23] K. Tanaka, M. Shionoya, *J. Org. Chem.* **1999**, 64, 5002.
- [24] L. Zhang, E. Meggers, *J. Am. Chem. Soc.* **2005**, 127, 74.
- [25] V. V. Mel'nikov, I. G. Il'ina, I. V. Fedyanin, B. N. Tarasevich, *Russ. J. Org. Chem.* **2008**, 44, 440.
- [26] A. A. Isaev, I. I. Lomovskii, K. G. Korolev, R. K. Karimov, *Chem. Heterocycl. Compd.* **2005**, 41, 1027.
- [27] V. G. Voronin, I. D. Petrova, A. N. Leksin, B. V. Shemeryankin, *Khim.-Farm. Zh.* **1976**, 10, 82.
- [28] G. A. Suboch, E. Y. Belyaev, *Patent No. RU 2154636* **2000**, Application No. RU1999.
- [29] I. R. Dunkin, A. A. El Ayeb, S. L. Gallivan, M. A. Lynch, *J. Chem. Soc., Perkin Trans. 2* **1997**, 1419.
- [30] M. L. Deda, M. Ghedini, I. Aiello, T. Pugliese, F. Barigelletti, G. Accorsi, *J. Organomet. Chem.* **2005**, 690, 857.
- [31] A. Rospigliosi, R. Ehlich, H. Hoerber, A. Middelberg, G. Moggridge, *Langmuir* **2007**, 23, 8264.

5. Summary

The first part of this dissertation (Chapter 1) describes the improved synthesis of photochromic spirobenzopyrans with use of ultrasonic irradiation. The successful preparation granted access to photoswitches that were equipped with hydroxyl, iodo, ethynyl and azido groups. The functional groups were then devised and differentiated during further progress to serve for the construction of functional π -systems and conjugation to biopolymers. The reversibility of the photoswitching was demonstrated for representative compounds, the absorption spectra of the corresponding merocyanines formed by photo-induced ring opening was discussed with respect to different substituents at the benzopyran moiety, and the solvatochromism of a spirobenzopyran that was equipped with a strong electron-withdrawing group was studied.

In the second part of this work (Chapter 2) the synthesis and optical properties of new photochromic dyads were described. The fluorescent building blocks (pyrene, perylene, nile red), bearing an alkyne-functionality, and a spirobenzopyran with an azido group were successfully prepared. CuAAC was used as a facile methodology for the assembly of the molecular dyads through 1,4-regioselective formation of 1,2,3-triazoles. Spectroscopic measurements revealed that by UV irradiation the spirobenzopyran unit of the dyads switch into their corresponding merocyanine form with appearance of its characteristic absorption band in the visible range. The fluorescence quenching was modulated by the formation of the photo-induced merocyanine form and was observed for all three dyads, indicating an energy transfer process from the fluorophore unit to the merocyanine. The efficiency of energy transfer increased from pyrene over perylene to nile red due to the enhanced spectral overlap.

Furthermore, the third part of this work (Chapter 3) reported on the synthesis and incorporation of a spirobenzopyran as an internal modification into oligonucleotides by two different strategies. A new spirobenzopyran building block for oligonucleotide synthesis has been effectually prepared. A useful synthetic prolongation and activation of the spirobenzopyran was introduced and conjugated with an acyclic linker system. The second strategy for incorporation of the spirobenzopyran modification was successfully performed with use of postsynthetic off-bead click ligation. Melting temperature measurements of the modified duplexes displayed significant destabilization compared to unmodified duplexes,

albeit photoinduced switching of the chromophore was not observed. Further investigations by time-resolved spectroscopy could bring about clarification on the photoprocesses.

The last part (Chapter 4) reported a novel and dexterous method for in situ azide formation and click conjugation of DNA with fluorescent labels. This approach completes the click repertoire by a complementary access and constitutes an alternative postsynthetic method for the covalent labeling of oligonucleotides on solid support. With use of CuAAC the ethynyl-modified chromophores (perylene, nile red) were conjugated with an azido group that was formed in situ by treatment of presynthesized oligonucleotides incorporating IdU with sodium azide on solid support. Methodically benefits are based on advantages of solid support synthesis and multifaceted implementations of functional π -systems. The characteristic properties of the nile red labeled DNA bearing a triazolyl bridge were studied and compared with a nile red labeled oligonucleotide with an ethynyl bridge. The results clearly display the great potential for the extensive use of this complete approach. Furthermore, in the last chapter the synthesis for new compositions of oligonucleotide building blocks was shown, specifically the synthesis and introduction of a quinolinol moiety into DNA for further purposeful metal-mediated applications as well as the synthesis and optical properties of a photoswitchable nucleoside.

6. Zusammenfassung

Der erste Teil der Dissertation (Kapitel 1) beschreibt die verbesserte Synthese von photochromen Spirobenzopyranen mittels Ultraschallbestrahlung. Die Methode wurde an Photoschaltern, die mit Hydroxyl-, Iodo-, Ethinyl- und Azid-Gruppen ausgestattet waren, erprobt. Diese zusätzlichen funktionellen Gruppen wurden im Verlauf weiterentwickelt und differenziert für den Aufbau funktioneller π -Systeme sowie für die Konjugation an Biopolymere verwendet. Die reversible Photoschaltung wurde für repräsentative Verbindungen nachgewiesen. Die Absorptionsspektren der durch licht-induzierten Ringöffnung gebildeten Merocyanine wurden in Hinblick auf die unterschiedlichen Substituenten am Benzopyran-Teil untersucht. Die Solvatochromie eines Spirobenzopyrans, das eine stark elektronenziehende Gruppe besitzt, wurde ebenfalls untersucht.

Im zweiten Teil dieser Arbeit (Kapitel 2) wurden die Synthese und die optischen Eigenschaften von neuen photochromen Dyaden beschrieben. Dafür wurde ein Spirobenzopyran mit einer Azid-Gruppe und die fluoreszierenden Bausteine (Pyren, Perylen, Nilrot), die mit Alkin-Funktionalitäten ausgestattet waren, erfolgreich synthetisiert. Anschließend wurde die Kupfer(I)-katalysierte Azid-Alkin-Cycloaddition (CuAAC) zur 1,4-regioselektiven Bildung von 1,2,3-Triazolen für den Aufbau der Dyaden verwendet. Die spektroskopischen Messungen zeigten anhand des Auftretens der charakteristischen Absorptionsbande im sichtbaren Bereich, dass durch UV-Bestrahlung die Spirobenzopyran-Einheiten der Dyaden in ihre entsprechende Merocyanin-Form umschalten. Die Fluoreszenzlöschung wurde durch die Bildung der photoinduzierten Merocyanin-Form moduliert und wurde bei allen drei Dyaden beobachtet, was auf Energie-Transfer-Prozesse von der Fluorophor-Einheit zum Merocyanin hinweist. Dabei stieg die Effizienz der Energieübertragung mit verbesserter spektraler Überlagerung an.

Im dritten Teil dieser Arbeit (Kapitel 3) wird über die Synthese und den Einbau eines Spirobenzopyrans als interne Modifikation in Oligonukleotide mittels zweier verschiedener Strategien berichtet. Dabei wurde ein neuer Spirobenzopyran-Baustein für die Oligonukleotid-Synthese erfolgreich synthetisiert. Eine nützliche synthetische Verlängerung und Aktivierung des Spirobenzopyrans wurde eingeführt und mit dem azyklischen Linkersystem konjugiert. Die zweite Strategie zum Einbau der Spirobenzopyran-Modifikation wurde erfolgreich unter Verwendung der postsynthetischen Off-bead Click Ligation

durchgeführt. Die Messungen der Schmelztemperaturen der modifizierten Duplexe zeigten eine erhebliche Destabilisierung im Vergleich zu den unmodifizierten Duplexen an, obgleich ein photoinduziertes Schalten des Chromophors nicht beobachtet wurde. Die weitere Untersuchung durch zeitaufgelöste Spektroskopie könnte zur Aufklärung der Photoprozesse beitragen.

Im letzten Teil (Kapitel 4) wird von einer neuartigen und geschickten Methode zur In-situ-Azid-Bildung und Click-Konjugation von DNA mit fluoreszierenden Label berichtet. Dieser Ansatz vervollständigt das Click-Repertoire durch einen komplementären Zugang und begründet eine alternative Methode für die kovalente Markierung von Oligonukleotiden auf der Festphase. Unter Verwendung der Kupfer(I)-katalysierten Azid-Alkin-Cycloaddition wurden Ethinyl-modifizierte Chromophore (Perylen, Nilrot) mit einer Azid-Gruppe konjugiert. Die Azid-Gruppe wurde in situ durch den Umsatz von vorsynthetisierten Oligonukleotiden, in welche IdU eingebaut war, mit Natriumazid auf der Festphase eingeführt. Die methodischen Vorteile basieren auf der Festphasensynthese und den vielfältigen Umsetzungen funktioneller π -Systeme. Die charakteristischen Eigenschaften der Nilrot-markierten und über eine Triazolyl-Brücke verknüpften DNA wurden untersucht und mit Nilrot-markierter DNA verglichen, bei der der Farbstoff über eine Ethinyl-Brücke verknüpft wurde. Die Ergebnisse zeigen klar das große Potential für die weitreichende Nutzung dieser Methode auf. Darüber hinaus wurde im letzten Kapitel die Synthese neuer Strukturen für Oligonukleotide-Bausteine vorgestellt, speziell die Synthese und der Einbau einer Quinolinol-Einheit in DNA für zielgerichtete metall-vermittelnde Anwendungen sowie die Synthese und optischen Eigenschaften eines photoschaltbaren Nukleosids.

7. Abbreviations

2D	two dimensional
4-NP	4-Nitrophenyl
4-NPC	4-Nitrophenylchloroformate
Å	Angström
A	absorption
Ac-dC	Acetyl Deoxycytidine
AMA	Ammonium hydroxide - Methylamine
a. u.	arbitrary units
BDF	Benzo[1,2- <i>b</i> :4,5- <i>b'</i>]difuran
BEMP	2- <i>tert</i> -Butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-Diazaphosphorin
Bz-dC	Benzoyl Deoxycytidine
calcd.	calculated
CD	circular dichroism
CDI	1,1'-Carbonyldiimidazole
CI	chemical ionisation
COSY	correlated spectroscopy
CPG	Controlled Pore Glass
CuAAC	Copper(I) catalyzed Azide-Alkyne Cycloaddition
δ	chemical shift
d	days
DCM	Dichloromethane
DCC	Dicyclohexylcarbodiimide
DEPT	Distortionless Enhancement by Polarisation Transfer
DIPEA	N,N-Diisopropylethylamine
4-DMAP	4-Dimethylaminopyridine
DMF	N,N-Dimethylformamide
DMSO	Dimethylsulfoxide
DMT	4,4'-Dimethoxytrityl
DNA	Deoxyribonucleicacid
dppf	1,1'-Bis(diphenylphosphino)ferrocene
ds	double strand

dU	2'-Deoxyuridine
EDC	1-ethyl-3-(3-dimethylaminopropyl) carbodiimide
EdU	5-Ethynyl-2'-deoxyuridine
EI	electron impact ionization
eq.	equivalents
ESI	electrospray ionization
EtOH	Ethanol
FAB	Fast Atom Bombardment
FC	Flash chromatography
GNA	Glycol Nucleic Acid
h	hours
HMBC	Heteronuclear Multiple Bond Coherence
HPLC	High Performance Liquid Chromatography
HPLC-DAD	High Performance Liquid Chromatography - diode array detection
HSQC	Heteronuclear Single Quantum Coherence
HV	high vacuum
Hz	Hertz
I	Intensity
IdU	5-Iodo-2'-deoxyuridine
IR	infrared
<i>J</i>	Coupling constant
K	Kelvin
λ	wavelength
LC	Liquid Chromatography
M	molar, mol/L
mbar	Millibar
m/z	Ratio mass / charge
mdeg	millidegree
MeCN	Acetonitrile
MeOH	Methanol
MHz	Megahertz
min.	Minutes
mM	millimolar
MMFF	Merck Molecular Mechanics Force Field

μM	micromolar
MS	mass spectrometry
Na-Pi	Sodium phosphate buffer
NBDF	3-Nitro-2-ethyldibenzofuran
NHS	N-Hydroxysuccinimide
nm	Nanometer
NMR	Nuclear Magnetic Resonance
NOE	Nuclear Overhauser Enhancement
NOESY	Nuclear Overhauser Enhancement Spectroscopy
PDB	Protein Data Bank
POM	Pivaloyloxymethyl
ppm	parts per million
R _f	Retention factor
RP	Reversed Phase
r.t.	room temperature
sec.	Seconds
ss	single strand
TBAF	Tetrabutylammoniumfluorid
TBAHS	Tetrabutylammoniumhydrogensulphate
TBDMS	tert-Butyldimethylsilyl
TBDMSCl	tert-Butyldimethylsilyl chloride
TBTA	Tris[(1-benzyl-1 <i>H</i> -1,2,3-triazol-4-yl)methyl]amine
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TINA	Twisted Intercalating Nucleic Acid
TIPDS	Tetraisopropyldisiloxane
TLC	Thin-layer chromatography
T _m	Melting temperature
TMS	Trimethylsilyl
TMSCl	Trimethylsilyl chloride
Ts	4-toluenesulfonyl
UV	Ultraviolet
Vis	Visible

8. Appendix

8.1. DNA supporting information

DNA synthesis was carried out with use of a PerSeptive Expedite 8909 synthesizer from Applied Biosystems (ABI). Reagents were purchased from Proligo, ABI and Glen Research. Controlled pore glass (500 Å) with loading capacity of 1 µmol was purchased from Proligo. Synthesized oligonucleotides were prepared with final trityl-off. A, G, T and C DNA building block phosphoramidites were prepared as 0.07 M solutions in acetonitrile (amidite diluent) with a standard coupling time of 40 s. Artificial (modified) DNA building blocks were prepared as 0.1 M solutions in acetonitrile (amidite diluent) and coupling time was extended to 375 s (see DNA synthesizer coding below).

Dblk: 3 % Dichloroacetic acid in dichloromethane; Wsh, Wsh A: Acetonitrile; Act: 0.45 M Tetrazole in acetonitrile; Caps: Acetanhydride in THF / pyridine (Cap A) and N-Methylimidazole in THF / pyridine; Ox: iodine in water / THF / pyridine.

Coding	Function	Mode	Amount/Arg1	Amount/Arg2
\$ Deblocking				
144	Index Fract. Coll.	NA	1	0
0	Default	WAIT	0	1.5
141	Trityl Mon. On/Off	NA	1	1
16	Dblk	PULSE	10	0
16	Dblk	PULSE	50	49
38	Diverted Wsh A	PULSE	40	0
141	Trityl Mon. On/Off	NA	0	1
38	Diverted Wsh A	PULSE	40	0
144	Index Fract. Coll.	NA	2	0
\$ Coupling				
1	Wsh	PULSE	5	0
2	Act	PULSE	5	0
23	6 + Act	PULSE	6	0
23	6 + Act	PULSE	1	75
2	Act	PULSE	4	300
1	Wsh	PULSE	7	525
1	Wsh	PULSE	8	0

\$ Capping

12	Wsh A	PULSE	20	0
13	Caps	PULSE	8	0
12	Wsh A	PULSE	6	15
12	Wsh A	PULSE	14	0

\$ Oxidizing

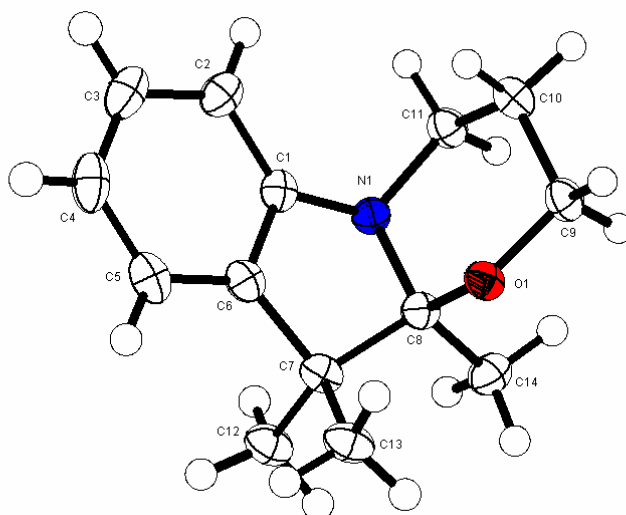
15	Ox	PULSE	15	0
12	Wsh A	PULSE	15	0

\$ Capping

13	Caps	PULSE	7	0
12	Wsh A	PULSE	30	0

8.2. X-ray crystal structure data

Crystals suitable for X-ray analysis were grown by slow evaporation of EtOAc at r.t. from a concentrated solution of the purified product. X-Ray structure of (S)-3,4,10,10a-Tetrahydro-10,10,10a-trimethyl-2*H*-[1,3]oxazino[3,2-*a*]indole.



Crystal data and structure refinement for (S)-3,4,10,10a-Tetrahydro-10,10,10a-trimethyl-2*H*-[1,3]oxazino[3,2-*a*]indole:

Empirical formula	C ₁₄ H ₁₉ N O
Formula weight	217.30
Crystal size	0.280 x 0.180 x 0.140 mm
Crystal description	prism

Crystal colour	colourless
Crystal system	Orthorhombic
Space group	P b c a
Unit cell dimensions	a = 11.0171(7) Å alpha = 90 deg. b = 8.4841(5) Å beta = 90 deg. c = 25.5815(14) Å gamma = 90 deg.
Volume	2391.1(2) Å ³
Z, Calculated density	8, 1.207 Mg/m ³
Absorption coefficient	0.075 mm ⁻¹
F(000)	944
Data Collection	
Measurement device type	STOE-IPDS diffractometer
Measurement method	rotation
Temperature	123(1) K
Wavelength	0.71073 Å
Monochromator	graphite
Theta range for data collection	3.13 to 25.87 deg.
Index ranges	-13 ≤ h ≤ 13, -10 ≤ k ≤ 10, -26 ≤ l ≤ 31
Reflections collected / unique	13152 / 2303 [R(int) = 0.0288]
Reflections greater I > 2σ(I)	1830
Absorption correction	None
Max. and min. transmission	0.989 and 0.979
Refinement	
Refinement method	Full-matrix least-squares on F ²
Hydrogen treatment	:
Data / restraints / parameters	2303 / 0 / 148
Goodness-of-fit on F ²	1.059
Final R indices [I > 2σ(I)]	R1 = 0.0387, wR2 = 0.0987
R indices (all data)	R1 = 0.0478, wR2 = 0.1020
Absolute structure parameter	.
Largest diff. peak and hole	0.267 and -0.160 e.Å ⁻³

Atomic coordinates and equivalent isotropic displacement parameters:

Atom	x/a [10^4]	y/b [10^4]	z/c [10^4]	$U_{eq.} / U_{iso.} [\text{\AA}^2 \cdot 10^3]$
O(1)	434(1)	3947(1)	5683(1)	25(1)
N(1)	-711(1)	2959(1)	6415(1)	22(1)
C(1)	251(1)	2797(1)	6779(1)	23(1)
C(2)	323(1)	3388(2)	7288(1)	29(1)
C(3)	1344(1)	2965(2)	7584(1)	36(1)
C(4)	2250(1)	2003(2)	7386(1)	37(1)
C(5)	2170(1)	1431(2)	6874(1)	33(1)
C(6)	1173(1)	1851(1)	6570(1)	26(1)
C(7)	812(1)	1343(1)	6021(1)	26(1)
C(8)	-200(1)	2590(1)	5894(1)	23(1)
C(9)	-278(1)	5373(1)	5667(1)	28(1)
C(10)	-759(1)	5809(1)	6206(1)	28(1)
C(11)	-1451(1)	4403(1)	6434(1)	26(1)
C(12)	275(1)	-337(2)	6060(1)	32(1)
C(13)	1856(1)	1352(2)	5623(1)	33(1)
C(14)	-1173(1)	2037(2)	5511(1)	30(1)

Anisotropic displacement parameters:

Atom	$U_{11} [\text{\AA}^2 \cdot 10^3]$	$U_{22} [\text{\AA}^2 \cdot 10^3]$	$U_{33} [\text{\AA}^2 \cdot 10^3]$	$U_{23} [\text{\AA}^2 \cdot 10^3]$	$U_{13} [\text{\AA}^2 \cdot 10^3]$	$U_{12} [\text{\AA}^2 \cdot 10^3]$
O(1)	27(1)	22(1)	27(1)	3(1)	5(1)	-1(1)
N(1)	22(1)	24(1)	22(1)	0(1)	2(1)	0(1)
C(1)	22(1)	23(1)	25(1)	5(1)	0(1)	-5(1)
C(2)	30(1)	33(1)	25(1)	1(1)	1(1)	-7(1)
C(3)	39(1)	43(1)	26(1)	6(1)	-6(1)	-15(1)
C(4)	30(1)	41(1)	40(1)	15(1)	-13(1)	-8(1)
C(5)	25(1)	29(1)	44(1)	9(1)	-1(1)	-1(1)
C(6)	25(1)	22(1)	31(1)	4(1)	2(1)	-4(1)
C(7)	25(1)	22(1)	30(1)	-1(1)	4(1)	0(1)
C(8)	25(1)	21(1)	23(1)	1(1)	4(1)	-3(1)

C(9)	31(1)	23(1)	31(1)	5(1)	2(1)	0(1)
C(10)	26(1)	22(1)	34(1)	1(1)	1(1)	2(1)
C(11)	22(1)	26(1)	28(1)	0(1)	2(1)	2(1)
C(12)	35(1)	23(1)	39(1)	-1(1)	5(1)	-2(1)
C(13)	30(1)	28(1)	40(1)	-4(1)	10(1)	1(1)
C(14)	33(1)	31(1)	26(1)	-2(1)	-1(1)	-4(1)

Hydrogen coordinates and isotropic displacement parameters:

Atom	x/a [10^4]	y/b [10^4]	z/c [10^4]	$U_{eq.} / U_{iso.} [\text{\AA}^2 10^3]$
H(2)	-294	4048	7427	35
H(3)	1416	3350	7932	43
H(4)	2927	1731	7597	45
H(5)	2786	766	6736	39
H(9A)	-969	5227	5424	34
H(9B)	229	6246	5532	34
H(10A)	-1306	6731	6178	33
H(10B)	-75	6093	6439	33
H(11A)	-1674	4632	6801	31
H(11B)	-2210	4239	6234	31
H(12A)	898	-1063	6190	38
H(12B)	3	-681	5713	38
H(12C)	-416	-332	6301	38
H(13A)	2257	2383	5628	39
H(13B)	1533	1147	5273	39
H(13C)	2444	531	5715	39
H(14A)	-1736	2905	5439	36
H(14B)	-1619	1149	5663	36
H(14C)	-787	1702	5184	36

8.3. List of Publications

Parts of this work are already published:

Christoph Beyer, Hans-Achim Wagenknecht,

“*In situ* azide formation and “click” reaction of nile red with DNA as an alternative postsynthetic route”, *Chem. Commun.* **2010**, 46, 2230-2231

Christoph Beyer, Hans-Achim Wagenknecht,

“Synthesis of Spiropyrans As Building Blocks for Molecular Switches and Dyads”, *J. Org. Chem.* **2010**, 75 (8), 2752-2755

Christoph Beyer, Hans-Achim Wagenknecht,

“Synthesis of DNA with Spirobenzopyran as an Internal Covalent Modification”, *Synlett* **2010**, 9, 1371-1376

8.4. Poster Presentations & Conferences

Vielberth-Symposium on Functional Nucleic Acids, Regensburg/Germany, **2009**.

Christoph Beyer, Janez Barbaric, Elke Mayer-Enthart, Hans-Achim Wagenknecht,

“DNA as a supramolecular architecture: Optical functionalization of oligonucleotides with fluorophores” (Poster), XVII International Roundtable on Nucleosides, Nucleotides and Nucleic Acids, Berne/Switzerland, **2006**.

4. Deutsches BioSensor Symposium, Regensburg/Germany, **2005**.

14. ANAKON, Regensburg/Germany, **2005**.

8.5. Curriculum vitae

Name: Christoph Beyer
Date of Birth: 19.02.1980
Place of Birth: Amberg
Nationality: German

Education

02/2006-03/2010	Dissertation: "Synthesis and characterization of photoswitchable building blocks based on spirobenzopyrans and new approaches for postsynthetic oligonucleotide labeling", Advisor: Prof. Dr. Hans-Achim Wagenknecht, Institute of Organic Chemistry, University of Regensburg
04/2005-12/2005	Diploma thesis: "Fluorescence imaging method for control of flux residues on soldered power transistors", Advisor: Prof. Dr. Otto S. Wolfbeis, Institute of Analytical Chemistry, Chemo- and Biosensors, University of Regensburg
10/2003-12/2004	Studies of Chemistry and Medicinal Chemistry (Diploma), University of Regensburg Exam: Diploma
10/2002-09/2003	Studies of Chemistry (Diploma), University of Regensburg
10/2000-09/2002	Studies of Chemistry (Diploma), Technical University of Munich Exam: Vordiplom
09/1991-06/2000	Kepler-Gymnasium Weiden i. d. Opf.; Exam: Abitur, majors in chemistry and mathematics

Research Experience

02/2006-current	Graduate student, Institute of Organic Chemistry, University of Regensburg (Advisor: Prof. Dr. Hans-Achim Wagenknecht)
01/2005-01/2006	Diploma student, Institute of Analytical Chemistry, Chemo- and Biosensors, University of Regensburg (Advisor: Prof. Dr. Otto S. Wolfbeis), collaboration with Infineon Regensburg

Teaching Experience

2006-2009	Teaching assistant in laboratory courses for chemistry, biology and biochemistry students
2005	Teaching assistant in laboratory courses for medicine students
10/2002-06/2003	Tutor in chemistry for students in medicine

Internships

08/1999-09/1999	Practical training, Max Planck Institute of Colloids and Interfaces, Golm/Potsdam, Prof. Dr. M. Antonietti
02/1999	Student trainee, Wacker, Burghausen
02/1998	Student trainee, Wacker, Burghausen